Unusual Abundance of Atypical Strains Associated with Human Ocular Toxoplasmosis

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To facilitate genotyping of *Toxoplasma gondii* in vitreous fluid of patients with severe or atypical ocular toxoplasmosis, polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) assays were developed for *SAG3* (p43) and *SAG4* (p18), 2 single-copy surface antigen genes. Together with strategies for *SAG1*, *SAG2*, and BI, multilocus RFLP analyses were performed on PCR-amplified parasite DNA present in 12 clinical specimens. Most samples (8/12) were not infected by type II or type III mouse-avirulent strains. Only 1 type III and 3 type II strains were identified, all from immunosuppressed patients. In 6 otherwise healthy adults and in 1 immunosuppressed patient, the *SAG1* allele associated with mouse virulence was amplified. Of 12 samples, 3 possessed true type I strains; 5 of 12 had new recombinant genotypes with alleles typical of type I or III strains at all loci examined. The unusual bias toward type I and/or recombinant genotypes bearing the *SAG1* type I allele associated with mouse virulence in immunocompetent adults has important implications for the epidemiology and efficacious treatment of ocular toxoplasmosis.

The protozoan parasite *Toxoplasma gondii* is widespread in nature with a high prevalence in many species of warm-blooded animals. Toxoplasmosis in humans is common and establishes itself as a lifelong chronic infection after consumption of undercooked meat harboring tissue cysts or from accidental ingestion of oocysts shed in cat feces. In immunocompetent adults, the disease is of variable severity, ranging from asymptomatic to influenza-like symptoms, lymphadenopathy, and ocular disease. When acquired congenitally or as a consequence of reactivation in immunocompromised patients, *T. gondii* can be fatal. Variation in the clinical presentation and severity of disease in susceptible persons has been attributed to several factors, including the genetic heterogeneity of the host and the genotype of the parasite responsible for infection [1–3].

The population biology of *T. gondii* indicates that, despite the existence of a well-described sexual cycle in cats, the parasite appears to reproduce in nature largely clonally, with sexual recombination occurring only extremely rarely. Of the 106 isolates genotyped to date at multiple loci, most (93) fall into 1 of only 3 distinct lineages: types I (17), II (52), and III (24) [4]. These 3 clonal types most likely represent remarkably successful genotypes for the niches so far examined—primarily diseased humans, domestic animals, and livestock. Rarely, strains with novel genotypes are isolated. On the basis of very limited analyses, the genotypes of the latter strains are consistent with their being naturally occurring recombinants of the 3 archetypal lineages, which suggests occasional mating [5]. These apparent recombinants presumably are representative of the total genetic diversity to be found in nature when isolates from unusual hosts or perhaps those that are asymptomatic or cause clinically atypical disease are eventually sampled and carefully analyzed.

Toxoplasmosis is one of the most common causes of infectious retinitis in both immunocompetent and immunosuppressed persons, accounting for 30%–50% of all cases of posterior uveitis in the United States [6, 7]. Until recently, toxoplasma retinochoroiditis in adults was believed to result mainly from periodic reactivation of latent cysts associated with congenital infection. Although this does occur, studies in Southern Brazil, France, and the United States indicate that chronic, postnatally acquired ocular toxoplasmosis in otherwise healthy adults is quite prevalent [8–10]. An outbreak of acute toxoplasmosis associated with municipal drinking water in Victoria, Canada, illustrates this point: ~20% of the 100 infected persons who experienced overt clinical symptoms went on to develop acute ocular disease as a result of the outbreak, which indicates a high rate of ocular involvement with acquired disease [11, 12]. Because the strains responsible for ocular toxoplasmosis have not been identified unequivocally, it has not been clear whether the genotype of *T. gondii* correlates with incidence, severity, or type of ocular disease. Type I strains,
however, have been implicated in several outbreaks associated with a high frequency of ocular toxoplasmosis [8, 13, 14].

Polymerase chain reaction (PCR) strategies for the detection of *T. gondii* DNA in intraocular fluid composed of vitreous and/or aqueous humor from patients with ocular disease have been applied only recently and have met with varying degrees of success [15–18]. Accurate genotyping with these methods has not been feasible, primarily because of a lack of optimized PCR restriction fragment length polymorphism (RFLP)–based assays for ocular fluid. This study identified 2 new loci that, together with previously published methods, allowed us to analyze 5 independent loci to genotypically identify strains of *T. gondii* that cause ocular disease in both immunosuppressed and immunocompetent adults with a clinical diagnosis of severe or atypical ocular toxoplasmosis.

**Materials and Methods**

**Clinical samples.** We studied banked vitreous fluid specimens obtained from 18 patients (18 eyes) with atypical and/or severe ocular inflammation during the course of diagnostic or therapeutic procedures performed from December 1993 through August 2000. Each patient eventually was found to have ocular toxoplasmosis, on the basis of clinical findings, response to treatment, histopathologic study, and/or PCR detection. Banked vitreous fluid specimens from an additional 21 inflamed eyes without a diagnosis (diagnostic dilemmas) and from 92 eyes with vitreoretinal disease not caused by *T. gondii* (64 with uveitis and 28 without inflammation) served as controls.

**PCR amplification.** All specimens were stored at or below −20°C until the assays were performed. Two nested primer pairs were used in all PCRs. Specimens were thawed at room temperature and were boiled for 10 min, and a small aliquot was taken to assay for *T. gondii* DNA by PCR. Some of the vitreous fluid specimens were ethanol precipitated before receipt, as described elsewhere [18].

For each gene, 2−5 μL of boiled vitreous or ethanol precipitate (depending on the total amount available) was subjected to PCR amplification by using external primers (ext), followed by nested amplification with the internal primers (int) with 1 μL of round 1 product as template. Both rounds used 5 μL of PCR buffer (10× Perkin-Elmer PCR buffer containing 15 mM MgCl2), 0.1 mM dNTP mix, 10 pmol of each primer, and 1.5 Unit of Taq DNA polymerase in a total reaction volume of 50 μL. Each of the 35 cycles consisted of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. We used 2 dilutions of a commercial preparation of RH strain (type I) *T. gondii* DNA (Advanced Biotechnologies) as strong- and weak-positive controls. In addition, in the strain typing assays, an aliquot of each genomic DNA from a type I (RH), type II (Prugniaud), and type III (CEP) strain were analyzed. One water (DNA-free) negative control was tested with each assay. Samples were amplified in an automated thermocycler (Perkin-Elmer GeneAmp PCR System 9700). The amplification products were visualized on an ethidium-stained 8% polyacrylamide gel.

**Genetic markers.** To determine the genotype of the *T. gondii* strain in the vitreous fluid samples, we used PCR to amplify gene products from 5 independent (4 were single copy) loci, each with distinctive RFLP patterns, to assign the allele present and ultimately the genotype of the infecting strain. The 5 genetic markers used in this study were *B1*, *SAG1*, *SAG2*, *SAG3*, and *SAG4*. Primer sequences and diagnostic RFLPs for *B1*, *SAG1*, and *SAG2* have been described elsewhere [4, 19–21]. In brief, the primers were as follows (all sequences in 5′–3′ orientation): Burg *B1*, forward (F)ext, GGAC-TGATCGTCCGTCACTGAG, Fint, TGCTAGGTTGGACTCAGTG-G, reverse (R)ext, TCTTTAAAGCGTTCGTTGTC, and Rint, GCCGACCAATTCGCGAATAACCC; Grigg *B1*, Fext, GTCTTCTGTCCTATTCGAC, Fint, TCTTCCAGCAGTCATTGC, Rext, ACGGATCGATTTCTTTCG, and Rint, CTGCAACATACGCTGTTGA; *SAG1*, Fext, GTTCTAACCACGCACCTCG, Fint, CAAATGTGCACCTGTAGGAAGC, Rext, GCATCAACAGTCCTGTGTG, and Rint, GCAAGAGGCAACATTGAAC; 5′-*SAG2*, Fext, GCTACCTCGAAAGCAACAC, Fint, GAAATGTTCAGGTTCCTGC, Rext, GCATCAACAGTCCTGTGTG, and Rint, GCAAGAGGCAACATTGAAC; 5′-*SAG3*, Fext, GCTTCTGTCCTATTCCAT, Fint, ATTCATCTCGTCTGCCTTG, Rext, TCAAGGGTGCAATATCGC, and Rint, AACAGTTGCAAGGACCAAC; *SAG4*, Fext, CAACCTCTACCATCCACCC, Fint, TCTTGTCCGTCTTTGCATTCA, Rext, GCAGTCTGTAGTTCAGAACA, and Rint, CACCAAGAGAGAGAGAAG; and *SAG4*, Fext, TACGATTTCAAGAGGGCT, Fint, GGACGACGTGA-AAACAGA, Rext, GTCTTCTCCGCTTGCTCGT, and Rint, CAGGAAGGTATGTTCTCTCTCTC. Primers for *SAG3* and *SAG4* were selected after DNA sequencing of 3 representative strains: type I (RH), type II (Prugniaud), and type III (CEP) revealed multiple polymorphic restriction endonuclease sites capable of identifying the allele present at these 2 loci. The *SAG3* PCR product was digested with either *NiaI*, *SacI*, or *AvaI*, to identify diagnostic restriction fragments (figure 1). Amplified *SAG4* product was digested with *SacI* or *Sau3AI* (figure 2).

**DNA sequencing.** For sequencing, PCR products were gel purified from low-melt agarose gels, followed by recovery on glass beads, using the UltraClean 15 DNA purification kit (MoBio Labs). Sequencing was done at the Stanford Protein and Nucleic Acids facility on 0.5–1.0 μg of purified DNA by using 2 pmol of the forward or reverse internal primers.

**Results**

**B1 gene assay.** Vitreous biopsy specimens submitted to our laboratory from 9 patients (9 eyes) clinically diagnosed as having atypical or severe ocular toxoplasmosis by the referring physician were first analyzed for the presence of *T. gondii* DNA by use of the nested PCR-based assay described by Burg et al. [19] for the detection of the *B1* gene. The *B1* gene is a 35-fold repetitive gene conserved in all *T. gondii* strains tested to date. *Toxoplasma* DNA was detected in 9 of 9 eyes, confirming the clinical diagnosis and providing a sensitivity of 100%. Controls used to check for contamination of reagents (2–5 μL of water diluted in PCR buffer and primers without vitreous material added) gave no detectable signal by this assay.

To test the specificity of the Burg *B1* gene assay for detection of *T. gondii* DNA, we analyzed vitreous biopsies from 92 control specimens and 21 diagnostic dilemmas. Of the 92 control speci-
mens, 0 of 64 from inflamed eyes and 2 of 28 from quiet eyes were positive for *T. gondii* DNA. Because no serologic information was available for the 2 *B1*-positive quiet eye controls, it could not establish whether these samples were false positives or indicated a latent but clinically asymptomatic *T. gondii* infection. Of the 21 specimens from diagnostic dilemmas, only 1 of 21 was positive for *T. gondii*, and the clinical picture for this patient is still evolving. These results indicate that the Burg *B1* assay when done on vitreous biopsy specimens is both highly sensitive and reliable (96% confidence) for the diagnosis of *T. gondii* infection in ocular disease, as reported elsewhere [16, 18].

**Strain typing.** To identify the *T. gondii* genotype responsible for ocular infection in the *B1*-positive vitreous samples, multilocus RFLP analyses and, for some samples, DNA sequencing of PCR-amplified parasite DNA were done to determine the allele present at multiple single copy loci. Subsequent to our initial study, we received 9 additional vitreous fluid specimens (3 raw vitreous and 6 vitreous DNA precipitates) determined by the submitting laboratory (Jack Remington [JSR], Palo Alto Medical Foundation, Palo Alto, CA) to be positive for *T. gondii* DNA by use of the Burg *B1* PCR assay. In total, 12 of the *B1*-positive clinical samples (our 9 and 3 from JSR) possessed sufficient parasite material to permit multilocus genotyping by the PCR-RFLP strategies described in the genetic markers section of Materials and Methods.

As shown in table 1, analysis by using previously described PCR-RFLP strategies for *SAG1* identified 4 of the 12 samples as having the allele common to types II and III. Surprisingly, 7 of the 12 samples amplified the relatively rare type I allele (1 sample, JSR 14, did not amplify at *SAG1*). PCR-RFLP analysis at *SAG2* tentatively discriminated between the 4 type II and III strains: 3 appeared to be type II and 1 appeared to be type III. For the 7 samples with a type I allele for *SAG1*, analysis at *SAG2* showed that 4 (179, 2033, JSR 02, and JSR 05) were of an unusual genotype and apparently possessed the type III, rather than the type I, allele at this latter locus (table 1). Thus, these results gave the first indication that strains 179, 2033, JSR 02, and JSR 05 possessed novel nonarchetypal genotypes.

The true extent of polymorphism between alleles cannot be fully assessed by RFLP analyses, so it was unclear whether the nonarchetypal strains found in samples 179, 2033, JSR 02, and JSR 05 in fact possessed a type I allele at *SAG1* and a type III allele at *SAG2* or if new alleles exist. Among archetypal strains, only 2 alleles have been described for the coding region of *SAG1* [22] (authors' unpublished data) and DNA sequencing of PCR-amplified *SAG1* from the nonarchetypal strains described here revealed no new allelic polymorphisms over 347 bp of the open reading frame.
Table 1. Clonal types assigned after analysis at 5 independent loci of amplified *Toxoplasma gondii* DNA from patients with ocular disease.

<table>
<thead>
<tr>
<th>Clonal type, patient</th>
<th>Immunosuppression before ocular toxoplasmosis</th>
<th>Clinical findings</th>
<th>Allele at indicated locus&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>2035</td>
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NOTE. HIV, human immunodeficiency virus; NA, not able to amplify product with polymerase chain reaction (PCR)-based assay at this locus; ND, not done because of insufficient material remaining.

<sup>a</sup> All patients were positive for *T. gondii* DNA by the *B1* gene PCR-based assay of Burg et al. [19].

<sup>b</sup> Alleles were scored on the basis of results of multiple restriction digests and/or DNA sequencing at each locus (details in Materials and Methods).

reading frame (i.e., all 4 were identical to the previously reported type I allele; data not shown). RFLP analyses previously identified only 3 alleles at *SAG2* [21], and sequence analysis of the *SAG2* PCR products established again that 179, 2033, JSR 02, and JSR 05 possessed the type III lineage allele over 241 bp of the open reading frame (data not shown). Of importance, only a single nucleotide polymorphism (SNP; conveniently identified by *Sau3A*I digestion) is responsible for the type I versus type III allele designation at the *SAG2* locus. Thus, these data alone do not make a compelling case for these strains possessing a new, apparently recombinant, genotype. Alternative explanations include that a chance mutation at the key nucleotide recently converted a type I allele to the type III sequence. Alternatively, there could be preferential PCR amplification of certain alleles for each locus so that a clinical isolate containing a mix of type I and III strains appears to contain only a single nonarchetypal and apparently recombinant (I/III) strain.

To address these alternative explanations, we needed to develop sensitive and highly specific PCR-RFLP strategies at additional polymorphic loci. This necessitated sequencing the alleles from previously established type I, II, and III lineage strains. For this study, we used the well described RH, Pru giaud, and CEP strains, respectively, as our reference types I, II, and III defining strains. Our efforts focused on 2 candidate genes, *SAG3* (p43) [23] and *SAG4* (p18) [24], which are single copy genes encoding *T. gondii* surface antigens. Sequence data for the *SAG3* gene in the 3 archetypal lineages identified 31 SNPs in the coding sequence that could be identified easily by RFLP (figure 1) or DNA sequence analysis (figure 3 and data not shown; GenBank accession numbers: RH-*SAG3*, AF340227; Prugniaud-*SAG3*, AF340228; and CEP-*SAG3*, AF340229). For *SAG4*, similar analyses revealed 15 SNPs in the 3 archetypal strains (data not shown; GenBank accession numbers: RH-*SAG4*, AF340224; Prugniaud-*SAG4*, AF340225; and CEP-*SAG4*, AF340226). Three of these SNPs gave convenient RFLPs (figure 2).

We selected nested primers to amplify short regions of DNA containing the most relevant polymorphisms, and product was readily amplified from control preparations containing as few as 5 parasites (data not shown). Nine unique nucleotide polymorphisms are bracketed by the *SAG3* internal primers (2 unique to type I, 5 unique to type II, and 2 unique to type III strains), whereas 14 nucleotide polymorphisms are encompassed by the *SAG4* internal primers (14 unique to type III strains; type I and II strains share the identical sequence). The *SAG3* locus is of note, since a single PCR amplification followed by digestion with *NdeI* discriminates between the 3 alleles (figure 1).

*SAG3* and *SAG4* PCR-RFLP analysis was done using the vitreous of the 12 clinical specimens (table 1). Despite repeated efforts, no *SAG3* or *SAG4* PCR product was amplified from
Figure 3. Sequence analysis at SAG3. Relevant polymorphic sites within a 225-nt SAG3 polymerase chain reaction (PCR) product among 13 geographically unrelated Toxoplasma gondii samples. Sequences are from 3 archetypal type I, II, and III strains (RH, Prugniaud, and CEP, respectively). Boxes: Unique nucleotide polymorphisms not shared by other 2 archetypal sequences; dots, identity with type I sequence. Sequencing results of 225-nt SAG3 PCR products derived from clinical samples: R VG 06 and R VG 08, identical to type I; 1039 and 2021, identical to type II; G23, 179, 2033, 2035, JSR 02, and JSR 05, identical to type III. JSR 14 and V92 specimens did not give amplifiable product at SAG3.

V92 vitreous, no SAG3 PCR product was amplified from JSR 14 vitreous precipitate, and we were unable to identify SAG4 PCR product in 2033 vitreous. We attribute these results to too little parasite DNA in the samples rather than to an absence of the SAG3 or SAG4 genes, because it was difficult to amplify product at other loci in these samples. RFLP analysis of the PCR products amplified from patients RVG 06, RVG 08, and JSR 14 confirmed that these specimens likely possessed true type I strains because type I patterns were seen at the 2 new loci. For samples 179, 2033, JSR 02, JSR 05, and JSR 05, identical to type III. JSR 14 and V92 specimens did not give amplifiable product at SAG3. RH-SAG3 sequence (GenBank accession no. AF340227) is essentially the same as that published for RH strain (accession no. L21720) except at 7 nt positions. Sequence agrees with RH strain ESTs (http://e2kroosdebug.cis.upenn.edu/est/toxo/) for this gene and for sequences of 2 clinical isolates. Differences in sequences in this report and that of Cesbron-Delauw et al. [23] could be due to unique polymorphisms in their RH strain, although sequence errors derived from their single isolate seem more likely to be the cause of discrepancies.

The strain responsible for infection in specimen 2035 was of special interest. It was initially assigned to the type I lineage after RFLP analysis at SAG1 and SAG2; however, it appears to be yet another novel recombinant possessing a type III allele at SAG3 and type I alleles at the other 3 loci. The possibility that these results are due to the presence of >2 strains infecting a single patient seems to be remote, because only 1 unambiguous signal was seen for all 4 single copy genes studied and, in mock analyses that used limiting amounts of genomic DNA, we found that a mixture of type I and III strains readily revealed the presence of both alleles (i.e., no preferential PCR amplification of 1 particular allele when DNA is limited; data not shown).

DNA sequencing directly on the 225 nt SAG3 and 269 nt SAG4 PCR products amplified from the clinical samples was next done to determine whether the RFLP analyses correctly predicted the allele present. As for SAG1 and SAG2, all the clinical samples were identical in their sequence polymorphisms to the allele type determined by RFLP analyses for SAG3 (figure 3) and SAG4 (data not shown). Of note, the JSR 14 SAG4 allele possessed 2 unique nucleotide polymorphisms in addition to the 14 SNPs that define a type I or II allele at this locus. This strain could be a true type I strain that has undergone a small amount of drift; however, because no other type I strain among 8 examined showed such polymorphism (authors’ unpublished data) and because we examined only 2 loci, it is entirely possible that this is a recombinant strain (in which greater polymorphism is more often seen) [14, 25].

We recently developed a PCR-RFLP genotyping strategy at the 35-fold repetitive B1 locus [20]. For samples with sufficient remaining material for PCR amplification, we performed B1 PCR-RFLP analysis. All samples genotyped at B1 as predicted on the basis of RFLP and sequence data accumulated for the 4 single copy genes (table 1). One control vitreous sample (JSR 03), determined by the submitting laboratory to be T. gondii DNA negative by Burg B1 PCR assay, on initial analysis was also negative by our B1 PCR-RFLP assay, but on receipt of more sample and retesting, it gave a positive result. The fact that JSR 03 was from a Toxoplasma-seronegative patient and that the sample was amplifiable only at the B1 gene is consistent with contamination, but the amplification product (type II or III) was not present in any of the other samples analyzed at that time, all the negative controls were clean, and patients with ocular toxoplasmosis having negative serum titers have been
described elsewhere [26, 27]. Insufficient material remained for
further testing, so the status of this sample remains unclear.

Discussion

We developed PCR-RFLP–based assays for allele typing of
Toxoplasma strains at 2 new loci, SAG3 and SAG4. With these
assays and by analysis of 3 other polymorphic loci, BI, SAG1,
and SAG2, in 12 isolates from patients experiencing severe or
atypical ocular toxoplasmosis, we found infection caused by
-type I in 3 isolates, type II in 3, and type III in 1, plus 5 novel
recombinant types. These findings were markedly different from
the expected frequencies, as discussed below. RFLP analysis at
any 1 locus would have misidentified the 5 recombinant strains.
Even analysis at 3 loci (e.g., SAG1, SAG2, and SAG4) would
have mistakenly identified the 2035 isolate as type I, when it
is another novel recombinant. Similarly, we cannot be sure that
the 7 strains that genotype here as types I, II, or III are not
novel recombinants until more polymorphic loci are identified
and the samples are analyzed further. Unfortunately, the limited
material available preclude such analyses on the samples stud-
iied here. Nevertheless, our results clearly reinforce the limita-
tions of typing based on only 1 or 2 loci, especially with isolates
sampled from exotic host species or geographically remote areas
or from patients who have differing disease presentations.

Studies of mice have shown that infection with the 3 lineages
of T. gondii results in dramatically different outcomes: Type I
strains are highly virulent, whereas types II and III are relatively
avirulent [13, 28]. As yet, there are few data for correlating the
strain of infecting parasite with the outcome of human disease.
In several studies, a substantial majority (~60%–80%) of symp-
tomatic infections in immunocompromised patients (e.g., those
with AIDS or lymphoma or transplant recipients) were of type
II parasites, but congenital infections were less uniform, although
type II dominated [4, 21, 29, 30]. Precise clinical findings were
not described, and no breakdown was given with regard to dis-
 ease outcome versus parasite type responsible for infection.

Our study focused on patients who presented with severe or
clinically atypical toxoplasmic retinochoroiditis. Although the
data set was limited, we saw a strong bias toward virulent type
I lineage or recombinant genotypes (8/12 isolates) rather than
of the type II expected, on the basis of environmental distri-
bution and of previous work with infected humans. Moreover,
in all 6 adults who were not immunocompromised (except per-
haps as a result of the treatment for the disease), the relatively
rare type I allele at SAG1 associated with mouse virulence was
amplified. The disproportionate number of recombinant geno-
types identified (5/12) was striking, and 4 appeared to be a
distinct and new clonal lineage, type IV. Previous studies iden-
tified only 3 of 106 strains as having recombinant genotypes
that possess alleles typical of type I and type III strains [4].
Thus, type I or these recombinant strains may be more likely
to result in severe ocular disease in humans. Whether the SAG1
gene or perhaps a nearby gene partially determines virulence
could not be determined by our limited study, but others have
noted that a region upstream of this gene appears to correlate
with severe disease in mice [5].

Support for a possible link between ocular disease and parasite
genotype is further strengthened by differences in the prevalence
of retinal involvement in different epidemics of acquired toxo-
plasmosis. In an outbreak in Atlanta in 1977, only 1 (3.6%) of
28 clinically symptomatic infected persons developed ocular le-
sions due to T. gondii infection [31]. However, in a 1995 outbreak
in Victoria, Canada, 20 (20.6%) of 97 persons had ocular disease,
a significantly greater proportion [12]. The strain responsible for
the Atlanta outbreak has not been identified, but the isolate
recovered from a patient in the Victoria outbreak was identified
as a type I strain [14]. Although this strain was not from a patient
who developed ocular toxoplasmosis, the epidemiology of this
well-defined outbreak suggests that it resulted from a single
source and thus, in all probability, a single strain [32]. In Erechim,
Brazil, where T. gondii seropositivity is endemic, ~18% (184/1042)
of the population have recurrent ocular toxoplasmosis [8]. Two
strains have been isolated from this region (OH3 and S11), and
both have been genotyped as mouse-virulent strains possessing
the type I allele at SAG1 [13].

Three reports document the isolation of live parasites from
immunocompetent adults experiencing acquired ocular toxo-
plasmosis; 2 isolates passaged in mice were virulent [33–35] and
therefore likely were type I or at least were not types II or III.
These results all support the notion that different strains cause
different disease outcomes in humans and that type I or type
I–like recombinant strains are more likely to be associated with
ocular disease in immunocompetent adults. This information
could substantially alter patient treatment, with more aggressive
monitoring and treatment of infections caused by more virulent
strains. This is important because the prevailing consensus is
that T. gondii infections in healthy adults are self-limiting and
thus clinically benign.

Ultimately, to interpret the significance of finding 1 strain
dominating in a given disease scenario, we will need strain
typing for the vast majority of human infection where Toxo-
plasma causes little if any disease. As yet, there is no informa-
tion on the strains that predominate in these infections and
such information likely will await the development of nonin-
vasive methods for strain typing (e.g., serology), as well as more
studies on the sources of human infection. For example, the
association of type II strains with symptomatic disease in
immunocompromised patients simply may reflect the prevalence
of type II infection in asymptomatic persons, which in turn
could derive from the abundance of type II infection in livestock
destined for human consumption [21] or the ability of type II
strains to form high numbers of cysts [3]. Alternatively, but less
likely, type I strains may be the dominant infection of humans,
which suggests that type II parasites are unusual for their ability
to cause disease in AIDS patients.
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References