Antibodies to *Toxoplasma gondii* in Individuals with First-Episode Schizophrenia

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We employed enzyme immunoassay (EIA) and Western blotting techniques to measure the level of antibodies to *Toxoplasma gondii* proteins in serum samples from 38 individuals undergoing their first episode of schizophrenia and from a group of matched control subjects. We found that the individuals with first-episode schizophrenia had significantly increased levels of IgG, IgM, and IgA class antibodies to *Toxoplasma* proteins, as compared with the control subjects.

Schizophrenia is a serious neuropsychiatric disease of uncertain etiology. Family and twin studies indicate that there is a strong genetic component to the risk of acquiring schizophrenia. However, epidemiological and neuropsychological studies have also indicated that some cases of schizophrenia may be associated with environmental factors, such as exposure to infectious agents. Specific infectious agents associated with the development of schizophrenia have not been identified [1].

*Toxoplasma gondii* is a protozoan parasite that infects a wide variety of warm-blooded vertebrates, including cats, livestock, and humans. Humans can become infected with *T. gondii* cysts after ingesting cat feces or undercooked meat [2]. Infection of a mother during early pregnancy can lead to infection of the fetus and development of a multisystem disease in the infant that can include severe mental retardation during childhood. Individuals infected later in fetal development or in the early postnatal period can have subclinical infection in which the parasite becomes encysted within the CNS. Reactivation of *T. gondii* after immunosuppression can lead to its persistent reactivation and replication in the brain and the ensuing development of neurological or psychiatric symptoms in some individuals [3, 4]. In animal models, infection with *T. gondii* can lead to altered behavior and changes in the levels of several neurotransmitters that are implicated in the pathogenesis of schizophrenia [5]. To investigate a potential association between *Toxoplasma* infection and schizophrenia, we examined the prevalence of antibodies to *T. gondii* in a group of individuals with first-episode schizophrenia and in a matched group of unaffected control subjects.

**Patients and methods.** As part of routine diagnostic procedures, serum samples were obtained from 38 participants in a structured neuroimaging study who were first hospitalized for schizophrenia, schizophreniform disorder, or schizoaffective psychosis by the Department of Psychiatry of the University of Heidelberg in 1998 or 1999. Clinical diagnoses were established by use of the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [6]. The median age of the patients was 27 years (range, 18–48 years). At the time of admission, none of the patients showed evidence of immunodeficiency or other immunologic abnormalities. None of the patients had evidence of a preexisting medical condition, of neurological disease, or of substance abuse. Serum samples were obtained from 27 healthy individuals, for use as controls. These control subjects were recruited in Heidelberg in 1999, screened for the absence of physical and psychiatric diseases, and matched by block design to the case patients according to sex, socioeconomic status, and age (±4 years). After matching, we verified that the case and control groups did not differ significantly with respect to these factors.

Blood samples were obtained from the patients by means of venipuncture at a mean (±SD) of 16 ± 10.2 days after the patients were first admitted to the hospital. Serum was separated from whole blood shortly after collection and was stored at −80°C until tested. All samples were tested such that the persons performing the assay were not aware of the identity of the patient.

We measured the levels of class-specific IgG, IgM, and IgA antibodies to purified *T. gondii* in the serum samples using EIA performed according to techniques described elsewhere [7]. The reagents for the *Toxoplasma* assays were obtained from Viro-Immun Labor-Diagnostika. In brief, microtiter wells were coated with antigens purified from the RH strain of *T. gondii*, into which was placed a 100-µL aliquot of serum diluted 1:
For qualitative determinations of reactivity for IgG and IgM class antibodies, a sample was considered to be reactive for *T. gondii* antigens if it yielded an arbitrary OD $>$0.4 for EIA. Rates of reactivity detected in the samples from case and control subjects were compared by use of Fisher’s exact test.

**Results.** Figure 1 shows the ODs generated by the reaction of serum samples to antigens of *T. gondii*. Although there was overlap of ODs for the 38 case patients and the 27 control subjects, the levels of antibodies measured in the serum samples from case patients, as a group, were significantly greater than the levels measured in the samples from control subjects: for IgG class antibodies, a sample was considered to be reactive for *T. gondii* antigens if it yielded an arbitrary OD $>$0.4 for EIA. Rates of reactivity detected in the samples from case and control subjects were compared by use of Fisher’s exact test.

Table 1. Comparison of class-specific reactivity to *Toxoplasma gondii* antigens, as measured by means of EIA and Western blotting, in serum samples obtained from individuals with first-episode schizophrenia (FES) and in samples obtained from control subjects.

<table>
<thead>
<tr>
<th>Class of antibody</th>
<th>No. (%) of samples reactive, by patient group</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients with FES ($n = 38$)</td>
<td>Control subjects ($n = 27$)</td>
</tr>
<tr>
<td>IgG</td>
<td>14 (36.8)</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>IgM</td>
<td>5 (13.1)</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>IgG or IgM</td>
<td>16 (42.1)</td>
<td>3 (11.1)</td>
</tr>
</tbody>
</table>

$^a$ Fisher’s exact test.
3 (11.1%) of 27 matched control subjects (P<.02; Fisher’s exact test). A total of 16 (42.1%) of 38 individuals with first-episode schizophrenia had either IgG or IgM class antibodies reactive to T. gondii proteins, compared with 3 (11.1%) of 27 control subjects (P<.007; Fisher’s exact test).

Discussion. Individuals with schizophrenia may differ from unaffected individuals with respect to the medications they have received and exposure to infectious agents that is related to hospitalization or lifestyle. However, the individuals with first-episode schizophrenia who participated in the present study had not been hospitalized previously and had been living in their usual environment before their initial hospitalization. They also did not show evidence of concurrent medical illnesses or drug exposure. Therefore, it is unlikely that the different levels of antibody to Toxoplasma proteins measured in the case patients and control subjects were related to incidental environmental exposures.

Because our study was cross-sectional, we could not determine when the antibodies to T. gondii were acquired in relation to the onset of psychiatric symptoms. We have not found evidence of increased levels of antibodies to T. gondii in other populations of individuals with chronic forms of schizophrenia, which indicates that differences between case patients and control subjects may be most marked at the beginning of the illness (R. H. Yolken and E. F. Torrey, unpublished observations). Additional cohort studies are in progress that are attempting to better define the relationship between T. gondii infection and the onset of the symptoms of schizophrenia and other psychiatric diseases.

The results of Western blotting confirmed the specificity of the IgG and IgM class antibodies to Toxoplasma proteins that were measured in the immunoassay system. (Western blotting was not performed for IgA class antibodies because neither defined reaction conditions nor control serum samples that tested positive for the antibodies were available.) However, the original antigenic stimulus for the antibodies to Toxoplasma proteins that were measured in these individuals could not be determined with certainty. Antibodies to Toxoplasma proteins can be produced during infection with T. gondii. However, some adults possess stable levels of antibodies to Toxoplasma proteins even though they have not had documented infection with this organism. These antibodies usually belong to the IgM class, but they may belong to the IgG class as well. The source of these “naturally occurring” antibodies is unknown but may be related to exposure to cross-reacting microorganisms [10, 11]. Additional studies should attempt to document infection with T. gondii in individuals with first-episode schizophrenia by use of methods such as the measurement of intrathecal antibody production [12] and the direct detection of Toxoplasma DNA by means of PCR analysis [13]. Prospective studies should also be directed at defining the temporal relationship between the acquisition of antibodies to T. gondii and the subsequent development of psychiatric diseases.

The replication of T. gondii can be inhibited by a number of different antiparasitic compounds. Several of the medications currently used to treat schizophrenia and other psychiatric diseases also have the ability to interfere with the replication of T. gondii organisms by modulating calcium-channel transport [14]. The establishment of an etiological link between infection with T. gondii and the onset of schizophrenia might provide a rationale for the development of new treatments for this devastating disease.

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