Shared Immune and Repair Markers During Experimental *Toxoplasma* Chronic Brain Infection and Schizophrenia

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Chronic neurologic infection with *Toxoplasma gondii* is relatively common in humans and is one of the strongest known risk factors for schizophrenia. Nevertheless, the exact neuropathological mechanisms linking *T gondii* infection and schizophrenia remain unclear. Here we utilize a mouse model of chronic *T gondii* infection to identify protein biomarkers that are altered in serum and brain samples at 2 time points during chronic infection. Furthermore, we compare the identified biomarkers to those differing between “postmortem” brain samples from 35 schizophrenia patients and 33 healthy controls. Our findings suggest that *T gondii* infection causes substantial and widespread immune activation indicative of neural damage and reactive tissue repair in the animal model that partly overlaps with changes observed in the brains of schizophrenia patients. The overlapping changes include increases in C-reactive protein (CRP), interleukin-1 beta (IL-1 β), interferon gamma (IFN γ), plasminogen activator inhibitor 1 (PAI-1), tissue inhibitor of metalloproteinases 1 (TIMP-1), and vascular cell adhesion molecule 1 (VCAM-1). Potential roles of these factors in the pathogenesis of schizophrenia and toxoplasmosis are discussed. Identifying a defined set of markers shared within the pathophysiological landscape of these diseases could be a key step towards understanding their specific contributions to pathogenesis.

Key words: *Toxoplasma gondii*/animal model/biomarker

Introduction

The etiology of schizophrenia remains poorly understood. The apparent discrepancy between strong evidence for having a first-degree relative with schizophrenia as a high relative risk (*RR* 5–12) factor¹ and the low effect size

of individual genes (*RR* 1.1–1.2) (reviewed in ref.²) has renewed interest in defining environmental risks associated with schizophrenia. Studies have attributed intermediate risk (*RR* 2–4) to sociogeographic factors (eg, immigration, urban residence) or exposure to infectious agents including bacteria, viruses, and protozoa. Collectively, those insights have spawned an integrated theory proposing that genetic factors such as immune susceptibility genes³ predispose individuals to increased risk of developing schizophrenia upon exposure to a trigger from the environment.⁴ Environmental components also have the potential to exacerbate disease. Nonetheless, precisely how environmental risk factors contribute to schizophrenia is unknown.

Among the infectious agents linked to schizophrenia, the intracellular protozoan *Toxoplasma gondii* has emerged as having one of the strongest associations (odds ratio 2.7) based on meta analyses of multiple studies.^{5,6} Chronic neurologic infection with *T gondii* is relatively common throughout human populations, with prevalence rates of 10%–50% based on serologic studies (reviewed in ref.⁷). Infection can be acquired prenatally or postnatally via exposure to a spore-like form derived from infected cats or consumption of infected meat. Acute infection is associated with minimal to moderate flu-like symptoms during dissemination and replication of proliferative tachyzoite stage parasites in lymphoid and other tissues. The immune response limits parasite replication but fails to eliminate the infection upon parasite differentiation into chronic bradyzoite stage parasites, which are encased in intracellular cysts within muscle cells and neurons. While substantiation of *T gondii* in the etiology of schizophrenia is incomplete, a plausible relationship is supported by the neuropathologic commonalities between these entities. *T gondii* infection

and schizophrenia both involve increased production of *L*-kynurenine and its metabolites affecting glutamate receptor signaling and neuro-excitotoxicity.^{8–10} *T gondii* infection and schizophrenia also both manifest significant neuroinflammation^{11–17} and dysregulation of neurotransmitters, particularly dopamine and serotonin.^{18–21} Additionally, several reports have documented the ability of *T gondii* to alter rodent neural connectivity²² and fear behavior,^{23,24} and influence human behavior (reviewed in ref.²⁵). Finally, *Toxoplasma* seropositive schizophrenic patients have been shown to score significantly higher on the PANSS-positive subscale^{26,27} and have reduced grey matter density compared with seronegative patients,²⁸ indicating that infection may be associated with exacerbated pathology. To our best knowledge, *T gondii* has not yet been detected in the brain of schizophrenia patients, but low infection levels often render it difficult to detect the parasite in tissues. Despite the emerging neurophysiologic and epidemiologic associations between *T gondii* infection and schizophrenia, no studies have evaluated and directly compared protein biomarker patterns of these diseases to identify additional commonalities.

Here we utilize a mouse model of chronic *T gondii* infection to identify biomarkers that are altered in serum and brain samples of mice at 2 time points during chronic infection. We also compare the identified biomarkers to those seen in “postmortem” brain samples from schizophrenia patients. Our findings suggest that *T gondii* infection causes substantial and widespread immune activation indicative of neural damage and, notably, reactive repair. The results further indicate that most of the biomarkers upregulated in schizophrenia brain tissue are also elevated in the brains of *T gondii* infected mice. The neuropathologic implications of shared biomarkers including interferon gamma (IFN γ), tissue inhibitor of metalloproteinases 1 (TIMP-1) and plasminogen activator inhibitor 1 (PAI-1), among others, are discussed.

Methods

Animal Handling and Infection

All procedures and protocols were carried out according to the Public Health Service Policy on Humane Care and Use of Laboratory Animals and Association for the Assessment and Accreditation of Laboratory Animal Care guidelines. The University of Michigan Committee on the Use and Care of Animals approved the animal protocol used for this study (Animal Welfare Assurance A3114-01, protocol PRO00004317). All efforts were made to minimize pain and suffering.

For infection experiments, brain cysts of the ME49 strain of *T gondii* (originally provided by Michael Shaw, University of Michigan) were maintained in donor female CBA/J mice (Jackson Laboratories). The brain was removed from a donor mouse 5 weeks post-infection, placed in 1 ml of sterile phosphate-buffered saline

(PBS), minced with fine scissors and homogenized by passage through a 20-gauge needle and syringe. Cysts were quantified by phase contrast microscopy. Recipient female CBA/J mice (10 mice per group) received either 18 cysts in 200 μ l sterile PBS by intraperitoneal injection or were left uninfected. The number of cysts was previously optimized to reliably infect brain.²⁹ Two or 6 months post-infection, mice were decapitated and exsanguinated to collect serum. Nine out of 10 mice survived in the 2-month infection cohort and 6 out of 10 mice survived in the 6-month cohort. All 10 uninfected mice survived both the 2-month and 6-month experiments, therefore 9 or 6 uninfected mice were randomly selected as control groups from the original uninfected cohorts. Brains were removed, washed in ice-cold PBS and placed on an ice-cold Petri dish. The frontal lobe was excised in the anterior one-third of the brain and immediately flash frozen in Lo-Bind Eppendorf tubes (Eppendorf) on dry ice/ethanol before transferring to -80°C .

Sample Preparation

Serum. The blood was allowed to clot at room temperature for 2 hours and centrifuged at 4000g for 5 minutes to remove particulate debris. The resulting supernatants were transferred to Lo-Bind polypropylene tubes and stored at -80°C until analyzed.

Brain Homogenate. Frozen tissue was homogenized using a Branson 4C15 sonicator in 9 volumes of ice-cold lysis buffer (50mM Tris-HCl pH 7.4 containing 0.7 μ g/ml pepstatin and complete Roche protease inhibition cocktail) in 2 cycles of 20 seconds at 20% amplitude. The resulting homogenate was centrifuged at 4 $^{\circ}\text{C}$ for 3 minutes at 10 000g and the supernatants were stored at -80°C in Lo-Bind tubes.

Multiplexed Immunoassay Analysis

Serum and brain homogenates from 2-month *T gondii* infection (250 μ l) were analyzed for levels of 88 markers (Rodent Antigens 2.0 MAP, Rat Metabolic 1.0 MAP and Rat Kidney MAP platforms), while brain samples from 6-month infection were analyzed for a subset of 60 markers (Rodent MAP) at the Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Myriad RBM). Rat Metabolic MAP and Rat Kidney MAP assays were not available at time of measuring the 6-month infection samples. The biomarker assay procedure has been described in detail previously.³⁰ Briefly, samples were analyzed at optimized dilutions and raw intensity measurements were converted into absolute protein concentrations using duplicate 8-point standard curves. Sample analysis was randomized to minimize bias due to measurement-related effects. Assay performance

was evaluated using quality control samples at low, medium, and high concentrations for each analyte.

Data Analysis

Biomarker data obtained from multiplexed immunoassay profiling was filtered to exclude analytes with large proportion of missing values. Markers with more than 30% of readouts outside the assay range in both of the groups (affected or control) were removed from further analysis. This resulted in exclusion of 19 analytes from the 2-month serum dataset, 20 analytes from the 2-month brain dataset and 16 analytes from the 6-month brain dataset. For the analytes remaining in analysis, readouts below the lowest limit of quantitation were replaced with half the minimum value measured for a given analyte, readouts above the upper limit of the assay range were replaced with twice the maximum value, and the remaining missing readouts (not measured due to insufficient quantity of sample) were replaced with the mean value for a given readout. Principal component analysis was used to determine presence of outliers. None was detected in any of the datasets using first and second principal components. Shapiro-Wilk tests showed that majority of the analyte levels were non-normally distributed, therefore nonparametric Wilcoxon rank-sum tests were used to compare affected and control groups. Fold changes (FCs) were calculated as a ratio between the mean value from the affected group and the mean value of the control group. *P* values were adjusted for false discovery rate using Benjamini-Hochberg procedure (*q* values).³¹ Significance of the overlap in changed analytes between different datasets was assessed using Fisher's exact test. For each comparison, only analytes measured in both datasets were considered in these analyses. Protein function was determined by uploading the UniProt Knowledgebase (UniProtKB) IDs of analogous human proteins into the Human Protein Reference Database. Pathway analysis was performed using the Ingenuity Pathway Analysis software (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity).

Comparison to Human Postmortem Brain

Data from multiplexed immunoassay profiling of *T. gondii* effects on mouse brain were compared to those from our previously published analysis of postmortem brain of schizophrenia patients.³² Brain tissue for this study (Brodman area 10) was obtained from the Stanley Medical Research Institute Array Collection. Full informed consent for tissue collection was obtained from first-degree relatives in accordance to the Declaration of Helsinki. The protocol was accepted by the Uniformed Services University of Health Sciences institutional review board (IRB) and local tissue use was approved by the Cambridgeshire 2 Local Research Ethics Committee. Approximately 100 mg of brain tissue was minced and homogenized in 9 volumes of ice-cold lysis buffer (50 mM

Tris-HCl pH 7.4 with Complete Mini Protease Inhibitor Cocktail tablets [Roche] and pepstatin). The resulting homogenates were transferred to Lo-Bind Eppendorf tubes and centrifuged for 3 minutes at 10 000g and 4°C. The supernatants were stored at -80°C until analyzed using Human Discovery MAP panel (Myriad RBM; for details see part Multiplexed Immunoassay Analysis). For consistency with the present study, human postmortem data were processed as described in section Data Analysis, log₁₀-transformed to approximate normal distribution and analyzed using stepwise logistic regression with Bayesian Information Criterion to adjust for potential confounding effects of age, gender, postmortem interval, brain pH, and alcohol use. Similarities to changes induced by infection with *T. gondii* in mice (2- and 6-month) were assessed only for those analytes that were measured in all 3 datasets.

Western Blotting

Thirty- to 50-mg pieces of snap frozen frontal lobe from the 6-month infected mice were transferred to the 1.5-ml microcentrifuge tubes and homogenized with disposable mini-pestles (Fisher) in 100 µl of lysis buffer (100 mM NaCl, 10 mM NaPO₄, 10 mM Na₄P₂O₇, 5 mM EDTA, 5 mM EGTA, 50 mM NaF, 2 mM Na₃VO₄, 1% [vol/vol] Triton X-100, 0.1% [wt/vol] SDS, pH 7.4). Samples were centrifuged at 16 000g 3 times, saving the supernatant as 80% of the total volume each time. Protein concentrations were assessed with a Bradford assay (BioRad). Uninfected and infected samples were pooled (within each group) and 80 µg total protein in SDS PAGE sample buffer with 1% 2-mercaptoethanol was loaded on a 12.5% SDS PAGE gel. Separated lysates were semi-dry transferred to Immobilon-FL membranes (Millipore), dried, rewetted with methanol, and either stained for 20 minutes with Ponceau S (0.2% [wt/vol] in 0.3% [wt/vol] trichloroacetic acid) or incubated for 30 minutes in blocking solution (5% [wt/vol] skim milk powder, 0.05% [vol/vol] Tween-20, 0.05% Triton X-114 in PBS pH 7.4). Westerns were performed by incubating blots with 1 µg/ml rabbit anti-mouse VCAM-1 (Cell Signaling) in antibody solution (1.25% [wt/vol] skim milk powder, 0.05% [vol/vol] Tween-20, 0.01% Triton X-114 in PBS pH 7.4) for 1 hour followed by 4 washes in 10.1% (wt/vol) skim milk powder, 0.05% (vol/vol) Tween-20 in PBS pH 7.4. Reactive bands were detected by incubation for 1 hour with horseradish peroxidase conjugated goat anti-rabbit (Jackson) in antibody solution, washing as above, incubation with enhanced chemiluminescence reagent (Pierce) and exposure to X-ray film.

Results

Effects of 2-Month *T. gondii* Infection on Serum Markers

Analysis of multiplexed immunoassay data from serum of mice following 2-month infection with *T. gondii*

revealed significant changes in levels of 40 markers ($P < .05$, Wilcoxon rank-sum test). Of these, 35 proteins were up-regulated and 5 proteins were down-regulated (figure 1). The highest FC was observed for C-X-C motif chemokine 10 (CXCL10; FC = 5.6, $P < .001$, $q = 0.003$) and the lowest FC was reported for interleukin 2 (IL-2; FC = 0.5, $P = .017$, $q = 0.049$). Half of the altered molecules (20/40), including 7 hits with the highest FC, were involved in immune response as referenced by the Human Reference Protein database. The second biggest group of significantly altered proteins (11/40) was related to cell communication. Detailed information about significant changes in the serum from 2-month *T gondii* infection can be found in supplementary table S1.

Effects of 2-Month *T gondii* Infection on Brain Markers

Following 2-month *T gondii* infection, 48 markers were significantly up-regulated and none of the proteins was

significantly down-regulated in the brain (figure 2a). The highest FC was observed for glucagon-like peptide 1 (GLP-1; FC = 177.3, $P = .001$, $q = 0.002$) and immunoglobulin A (IgA; FC = 93.6, $P < .001$, $q = 0.001$). Out of 48 altered molecules, 21 were related to immune function and 18 were involved in cell communication (detailed description in supplementary table S2). Biological functions linked to these changes are shown in figure 3. There was a significant overlap between changes induced by 2-month *T gondii* infection in serum and brain ($P = .020$; figure 4a), with 27 analytes consistently altered in both tissues.

Effects of 6-Month *T gondii* Infection

Chronic, 6-month *T gondii* infection in mice significantly affected levels of 37 brain markers (figure 2b). Proteins with the highest FC, myeloperoxidase (FC = 148.1, $P = .005$, $q = 0.007$) and TIMP-1 (FC = 62.9, $P = .004$, $q = 0.007$), were involved in metabolism/energy pathways and cell

Fig. 1. Biomarkers in the serum significantly altered by chronic 2-month *Toxoplasma gondii* infection in mice. Only proteins which met $P < .05$ cutoff (Wilcoxon rank-sum test) in any of the groups (2- or 6-month infection) and sample type (serum or brain) are shown for ease of comparison. Fold change was calculated as a ratio between the means of the affected and control groups. Primary protein function was determined using the Human Protein Reference Database and color-coded in the chart. Proteins shown in grey were not measured or excluded from analysis due to high proportion of missing values.

Fig. 2. Biomarkers in the brain significantly altered by chronic 2-month (a) and 6-month (b) *Toxoplasma gondii* infection in mice (for details see description of figure 1). Asterisks indicate proteins significantly changed in postmortem brain of schizophrenia patients (refer to figure 5).

growth and maintenance, respectively. Since neutrophils express high levels of myeloperoxidase, elevation of this marker may suggest neutrophil infiltration into the brain. The only down-regulated marker was insulin (FC = 0.5, $P = .049$, $q = 0.058$). Among all significant markers, 16 were immune-related and 12 mediated cell communication (supplementary table S3). A total of 60 common molecules were measured in both 2-month and 6-month brain samples. Thirty-one of them showed consistent increase in both groups and this overlap was significant ($P = .009$; figure 4b). While protein changes in brain at 2 months indicated damage of the central nervous system and have been linked to increased apoptosis and reduced myelination, markers of these processes diminished by 6 months (figure 3). However, proteins associated with residual central nervous system damage were still significantly altered after 6-month infection. Mice infected for 6 months harbored an average of 1767 cysts per brain and weighted significantly less than control animals at the end of the study (data on body weight was not available for mice infected for 2 months).

Comparison to Schizophrenia Brain

Schizophrenia ($N = 35$) and control human brain samples ($N = 33$) were matched for age, gender, race, alcohol consumption, brain weight and postmortem interval (demographic details are shown in supplementary table S4). There were significant differences in brain pH ($P = .017$), suicide status ($P = .011$), and smoking ($P = .006$) between patients and controls, although the latter was not recorded for 23 participants. A total of 52 common proteins were measured in human samples and both 2- and 6-month *T. gondii*-infected animals. After

adjusting for relevant covariates, 7 of these proteins were significantly increased and 2 of them were significantly decreased in schizophrenia brain (supplementary table S5). These included 6 brain markers of *T. gondii* infection identified previously in mice: C-reactive protein (CRP), IL-1 beta (significant only for 2-month infection), interferon gamma (IFN γ), PAI-1 (significant only for 6-month infection; not detected in 2-month group), TIMP-1 and vascular cell adhesion molecule 1 (VCAM-1; figures 4c and 5). The overlap between changes detected in the animal model and human brain was not significant ($P = .431$ for schizophrenia vs 2-month infection comparison and $P = 1.000$ for schizophrenia vs 6-month infection comparison, figure 4c). Available serology data indicated that 3 patients tested positive for anti-*T. gondii* antibodies.

Validation of Principal Biomarkers

Increased levels of VCAM-1 in pooled animal brain extracts after 6 months of infection were validated by Western blot (figure 4d). The remaining proteins significantly altered in the animal models that overlapped with changes observed in the brain of schizophrenia patients (CRP, IFN γ , PAI-1, and TIMP-1) could not be reliably detected in either the control or disease tissue using Western blot analyses, despite testing different amounts of brain lysate, several antibodies for most of the biomarkers, and employing different methods of detection.

Discussion

Although a link between *T. gondii* chronic infection and schizophrenia is well documented,^{5,6} the question of cause

Fig. 3. Biological functions altered in the mouse brain by chronic *Toxoplasma gondii* infection. The heat map shows function-specific activation z scores calculated with the Ingenuity Pathway Analysis software from individual fold changes of significantly altered proteins. Biological functions with the highest total z score across the groups are sorted to the top.

or effect remains unknown. The molecular underpinnings of this association are also poorly understood. In the present study, we identified shared alterations of several molecular factors in the brains of schizophrenia patients and mice chronically infected with *T. gondii*. While studies have examined global changes in mRNA expression^{33,34} or alterations in the levels of specific proteins³⁵ in chronically infected mouse brain, the current report is the first to investigate changes in host protein expression during experimental *T. gondii* infection using a proteomics approach. Moreover, comparison to protein changes in the prefrontal cortex of schizophrenia brains with those from the frontal lobe of chronically infected mice revealed a distinct set of markers, suggesting common alterations

in immune activation (VCAM-1, CRP, IFN γ) and tissue repair (TIMP-1, PAI-1). Interestingly, these results concur with gene expression data, because increased expression of VCAM-1, IFN γ , TIMP-1, and PAI-1 was recently reported in BALB/c mice chronically infected with *T. gondii* strain ME49.³⁴ CRP gene expression was not detected in this recent study, presumably because it is mainly expressed and synthesized in the liver and then secreted into the blood stream.³⁶ It should be noted that the CBA/J mouse strain used for this study is susceptible to high parasite burden during chronic infection, which likely exacerbates the inflammatory immune response.^{37,38} Nonetheless, it was previously suggested that the CBA/J model is a suitable model based on the notion that limited reactivation of *T. gondii* underlies neurologic complications of nonlethal infection.³⁵

A strong pro-inflammatory response to *T. gondii* infection (and possibly other pathogens, such as neurotropic viruses) in some patients may contribute to schizophrenia etiopathogenesis through several mechanisms. IFN γ produced by T cells, natural killer cells infiltrating the brain, and by resident microglia,³⁹ is considered the main mediator of the immune response against *T. gondii*.⁴⁰ It primes phagocytes to produce toxic intermediates that suppress the growth of the parasite, but also increases the activity of indoleamine 2,3-dioxygenase.⁴¹ This enzyme degrades tryptophan, which is necessary to maintain replication of *T. gondii* in its active (tachyzoite) state, to kynurenine. Interestingly, imbalance in the kynurenine pathway of tryptophan degradation has been linked to schizophrenia. It is hypothesized that increased conversion of tryptophan to kynurenine acid by astrocytes leads to inhibition of *N*-methyl-d-aspartate (NMDA) receptors and results in hypoglutamatergic neurotransmission in schizophrenia.⁹ Kynurenate is also toxic to oligodendrocytes via an as yet undefined mechanism that is unrelated to NMDA⁴² and may therefore contribute to demyelination processes in schizophrenia. The kynurenine pathway constitutes a promising target for novel antipsychotic drugs. However, a recent study revealed that kynurenine pathway alterations in acutely *T. gondii*-infected mice might not fully reflect changes observed in the brain of schizophrenia patients,¹⁰ suggesting that other mechanisms may contribute to schizophrenia pathogenesis in individuals infected with *T. gondii*.

Such mechanisms could involve structural modifications of central nervous system tissue caused by infiltrating lymphocytes. IFN γ is known to induce expression of VCAM-1 in endothelial cells to allow recruitment of cytotoxic T cells to the site of *T. gondii* infection.⁴³ This is associated with the formation of collagen-like structures that guide lymphocyte migration.⁴⁴ Also excessive cytokine responses to *T. gondii* infection may cause neuronal apoptosis and glial damage, decreasing the neurotrophic support and inducing structural changes.⁴⁵ In line with these findings, changes observed in our study were

Fig. 4. Overlap between proteins altered by *Toxoplasma gondii* infection in mouse and biomarkers of schizophrenia. (a) Overlap between serum and brain markers altered in mice by 2-month infection with *T. gondii*. (b) Overlap between brain markers changed by chronic, 2- and 6-month *Toxoplasma* infection in mice. (c) Overlap between brain markers in 2 *T. gondii* models and schizophrenia. Significance of the overlap (P value) was calculated using 2-tailed Fisher's exact test of only those analytes, which were measured in all relevant datasets. Arrows indicate the direction of change (↑ up- or ↓ down-regulated). (d) Western blot validation of VCAM-1 in pooled mouse brain extracts after 6 months of infection. Left panel shows staining for mouse VCAM-1 and right panel shows total protein staining with Ponceau S to assess loading. Abbreviations: UI, uninfected; I, infected.

indicative of central nervous system tissue damage, demyelination and increased apoptosis, in particular 2 months after infection (figure 3). These findings are potentially relevant to the previously reported decrease in grey matter density of *Toxoplasma* seropositive schizophrenia patients compared with seronegative patients.²⁸ Also, alterations in synaptic, dendritic and axonal organization have been linked to macroscopic features observed in the brain of schizophrenia patients, such as decreased cortical volume.⁴⁶ Myelin abnormalities have been described in schizophrenia and linked to abnormal neural connectivity and functional impairment.⁴⁷

Our finding that levels of CRP are increased in the brain of schizophrenia patients adds to the evidence of activated immune response in schizophrenia. CRP is a protein involved in acute phase response and is considered

a generic marker of inflammation. Previous studies have shown increased levels of peripheral CRP in schizophrenia that was independent of potential confounding factors such as body mass index or smoking.⁴⁸ One study has found a positive association between serotiters to *T. gondii* infection and circulating CRP levels in schizophrenia.⁴⁹ Levels of CRP remain elevated regardless of treatment with antipsychotic medications⁵⁰ and are associated with the severity of cognitive impairment,⁵¹ suggesting that interventions directed at lowering CRP levels could benefit schizophrenia patients.

Immune activation-induced damage and tissue remodeling processes during *T. gondii* infection in the brain are controlled by matrix metalloproteinases, enzymes that degrade extracellular matrix proteins, as well as their inhibitor TIMP-1 produced by astrocytes and

Fig. 5. Brain markers of *Toxoplasma gondii* infection significantly altered in postmortem brain of schizophrenia patients. Plots show individual levels of given analytes in schizophrenia (SCZ) and control brain samples (CTRL) as well as in infected (+) and control (–) animal groups following 2- and 6-month *T. gondii* infection. Imputed values are shown in grey and horizontal lines represent the mean. * $P < .05$; ** $P < .01$; *** $P < .001$.

microglia.⁵² Our study showed increased TIMP-1 levels in both animal models and schizophrenia. Overexpression of TIMP-1 is known to impair long-term potentiation (LTP) in the prefrontal cortex.⁵³ Extracellular matrix abnormalities and resulting LTP-like plasticity deficits are likely to contribute to the pathophysiology of schizophrenia and result in impaired information processing in patients.^{54,55} Antipsychotic drugs are known to normalize extracellular matrix abnormalities in schizophrenia through epigenetic regulation.⁵⁵

The exact role of PAI-1 in *T. gondii* infection and schizophrenia-related mechanisms is unknown. Similar to TIMP-1, PAI-1 controls the matrix metalloproteinase activity and degradation of extracellular matrix proteins. However, excessive PAI-1 may lead to accumulation of collagen and scar formation.⁵⁶ Increased levels of PAI-1 may also be linked to reduced neurotrophic support in the brain of schizophrenia patients, because PAI-1 is involved in brain-derived neurotrophic factor maturation in the hippocampus.⁵⁷ Nevertheless, further studies are required to investigate the exact impact of elevated PAI-1 on brain function.

One of the limitations of the present study involves the serological status of schizophrenia patients whose brains were analyzed. Using available data, we confirmed that 3 of these patients were positive for anti-*T. gondii* antibodies. However, this number was too low to allow meaningful statistical analysis. Therefore, it was impossible to determine the relationship between the identified markers and *T. gondii* infection in the patient samples. It is

likely that other infections such as those with neurotropic viruses may induce similar changes, in particular in the context of the findings that viral infections may increase the risk of schizophrenia.^{58,59} Nevertheless, these considerations do not invalidate the neuropathological implications that different disease etiologies (both infectious and noninfectious) converge and present with changes in similar disease markers.

In summary, this study reveals a distinct subset of immune and repair markers that are shared during experimental chronic toxoplasmosis and in schizophrenia. Due to restraints in direct detection of *T. gondii* cysts in the brain of immunocompetent individuals, measurement of surrogate protein markers provides a promising alternative for investigating *T. gondii* infection in mice and humans. These findings will serve as the basis for future studies focused on delineating common molecular and cellular mechanisms underlying the pathobiology of chronic toxoplasmosis and schizophrenia.

Supplementary Material

Supplementary material is available at <http://schizophreniabulletin.oxfordjournals.org>.

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