BRIEF COMMUNICATION

Toxoplasma-Specific IgG Subclass Antibody Response in Cerebrospinal Fluid Samples from Patients with Cerebral Toxoplasmosis

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SUMMARY

Cerebral toxoplasmosis can be highly debilitating and occasionally fatal in persons with immune system deficiencies. In this study, we evaluated the Toxoplasma gondii-specific IgG subclass antibody response in 19 cerebrospinal fluid (CSF) samples from patients with cerebral toxoplasmosis who had a positive IgG anti- T. gondii ELISA standardized with a cyst antigen preparation. There were no significant differences between the rates of positivity and the antibody concentrations (arithmetic means of the ELISA absorbances, MEA) for IgG1 and IgG2, but the rates of positivity and MEA values for these two IgG subclasses were significantly higher than those for IgG3 and IgG4. The marked IgG2 response in CSF from patients with cerebral toxoplasmosis merits further investigation.

KEYWORDS: Cerebral toxoplasmosis; Cerebrospinal fluid; IgG subclasses.

INTRODUCTION

Human infection with Toxoplasma gondii is usually asymptomatic or is associated with mild, non-specific clinical symptoms in the majority of immunocompetent persons. However, toxoplasmosis can be highly debilitating and occasionally fatal in persons with immune system deficiencies and in congenitally infected infants.1-3,10,13,16

Host resistance to T. gondii is predominantly controlled by cell-mediated immunity, although the humoral immune response may also play an important role.4-7,11,12,17-19,25,26 In humans, the major antibody class produced in the humoral response to T. gondii is IgG3. Studies based on immunoenzymatic techniques (ELISA, immunoblot) standardized with tachyzoite antigen preparations have shown that IgG1 is the dominant IgG isotype involved in humoral response to T. gondii in humans.2,5,10,13,17,18

Determination of the IgG subclass antibody response to T. gondii could contribute to our understanding of the pathogenesis of toxoplasmosis, as well as its diagnosis.5 The cyst stage represents a lifetime risk for the reactivation of Toxoplasma infection in immunocompromised individuals.11,16 The aim of this study was to evaluate the Toxoplasma-specific IgG subclass antibody response in cerebrospinal fluid (CSF) samples from patients with cerebral toxoplasmosis who had a positive IgG anti-T. gondii ELISA standardized with a cyst antigen preparation.

MATERIAL AND METHODS

Patients and samples: Toxoplasma-specific IgG subclasses were evaluated in CSF samples from 19 patients with cerebral toxoplasmosis who were positive for anti-T. gondii IgG in CSF by ELISA, using the cyst antigen preparation described below. All patients had clinical and neuroimaging findings compatible with cerebral toxoplasmosis and the brain lesions and symptoms improved after anti-parasitic treatment. The CSF samples of ten patients had a positive nested polymerase chain reaction using primers for the B1 gene. Twenty-five CSF samples from patients with other neurological disorders [multiple sclerosis (n = 10), neurocysticercosis (n = 5), neurosyphilis (n = 3), neurocryptococcosis (n = 3) and bacterial meningitis (n = 4)] were used as controls. The CSF samples from patients with cerebral toxoplasmosis and other neurological disorders were collected for diagnostic purposes. After completion of all the solicited routine tests, the remaining volume of the CSF samples were used to detect Toxoplasma-specific IgG subclasses. All patients were attended to the university hospital of the State University of Campinas (UNICAMP), Campinas, Sao Paulo, Brazil. This study was approved by the Ethics Committee of the Faculty of Medical Sciences, UNICAMP, in accordance with the resolutions of the Brazilian National Ethics Committee.

Antigen preparation: cysts from the P strain of T. gondii were purified using the procedure described by KASPER (1989) from brains.

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ELISAs: serial dilution experiments were performed to determine optimal concentrations of reagents (antigen, monoclonal antibodies and conjugate) to be used in the ELISAs. The wells of polystyrene microtiter plates (Greiner Bio-One, Kremsmünster, Austria) were coated with antigen preparation (1 µg protein/mL in 0.1 M carbonate/bicarbonate buffer, pH 9.5) by incubating for one h at room temperature (RT) and 16 h at 4 °C. After incubation, the wells were washed three times with PBS containing 0.1% Tween 20 (PBS-T) and, after that, 100 µL of PBS-T containing 0.1% bovine serum albumin were added to the wells. After 30 min incubation at RT, the wells were washed twice with PBS-T and 100 µL of each CSF sample diluted 1:5 with PBS-T were added in duplicate for the wells for one h at RT. Subsequently, the wells were washed three times with PBS-T and 100 µL of monoclonal anti-human IgG1, anti-human IgG2, anti-human IgG3, or anti-human IgG4 (Sigma, St. Louis, MO, USA) diluted 1:750 in PBS-T were added to each well and the plates were incubated for one h at RT. After washing the wells three times with PBS-T, 100 µL of conjugate (peroxidase-labeled sheep anti-mouse IgG; Sigma) diluted 1:1000 in PBS-T were added to the wells and the plates were incubated for one h at RT. After incubation and three washes with PBS-T, 100 µL of substrate (0.42 mM tetramethylbenzidine and 1.42 mM H2O2, in 0.1 M sodium acetate/acetic acid buffer, pH 5.5) were added to the wells. Ten minutes after substrate addition, the reactions were stopped by adding 50 µL of 2 N H2SO4 to each well and the resulting absorbances were read at 450 nm in a Multiskan ELISA reader (Labsystems, Helsinki, Finland). Positive and negative controls were included in each plate. Each CSF sample was tested in duplicate and the mean absorbance determined. The final absorbance for each CSF sample was determined by subtracting the mean absorbance of two antigen controls in the corresponding plate. The cut-off value for each assay was determined by a receiver operating characteristic (ROC) curve using the ELISA results obtained with CSF samples from patients with cerebral toxoplasmosis and other neurological disorders.

Data analysis: the rates of positivity and arithmetic means of the ELISA absorbances (MEA) for each subclass were compared using the Cochran Q test and repeated measures ANOVA with transformation by ranks and profile test, respectively, with p < 0.05 indicating significance. The statistical analyses were done using SAS (Statistical Analysis System) for Windows version 9.2 (SAS Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Table 1 shows the rates of positivity of the ELISAs for the detection of IgG subclasses, as well as the MEA values for CSF samples from patients with cerebral toxoplasmosis. There were no significant differences between the rates of positivity and the MEA values for IgG1 and IgG2, but the rates of positivity and MEA values for these two IgG subclasses were significantly higher than those for IgG3 and IgG4 (p < 0.0001, respectively). The IgG1 and IgG2 ELISAs showed the same rate of positivity, but the MEA of the IgG1-ELISA was significantly higher than that of the IgG2-ELISA (p < 0.0001).

Table 1

| IgG subclass | Rate of positivity (%) | MEA       | p          |
|--------------|-----------------------|-----------|------------|
| IgG1         | 84.2 (a)              | 0.163 (c) | p = 0.0042*|
| IgG2         | 73.7 (a)              | 0.264 (c) | p = 0.0042*|
| IgG3         | 36.8 (b)              | 0.074 (d) | p < 0.0001**|
| IgG4         | 36.8 (b)              | 0.041 (e) | p < 0.0001**|

MEA = mean of the ELISA absorbances; *Cochran’s Q test; **Repeated measures ANOVA and profile test. Values followed by the same letter are not significantly different.

Infection with T. gondii is controlled primarily by cell-mediated immunity. Some studies have also shown that the humoral immune response may protect against the parasite. Several studies using immunological techniques standardized with tachyzoite antigen preparations have shown that IgG1 is the dominant IgG subclass involved in the humoral response to T. gondii in humans.

The rupture of Toxoplasma cysts in the brain may cause disease reactivation and severe encephalitis in immunocompromised hosts. However, there is only limited data on the utility of detecting Toxoplasma-specific antibodies in CSF samples from patients with cerebral toxoplasmosis. POTASMAN et al. (1988) showed that production of anti-T. gondii IgG antibodies in the central nervous system may be diagnostic of toxoplasmic encephalitis. CHANDRAMUKHI (2004), using a commercial ELISA kit (Omega Diagnostics, UK), detected Toxoplasma-specific IgG antibodies in 92% of CSF samples from autopsies of proven cases of cerebral toxoplasmosis, indicating that the detection of specific antibodies in CSF can be a useful adjunct to clinical and neuroimaging findings for the diagnosis of this neuroinfection. MEIRA et al. (2011) showed that the detection of Toxoplasma-specific IgG antibodies in CSF samples by immunoenzymatic techniques standardized with T. gondii excreted/secreted antigens (ESA), in association with clinical, serological and radiological information, can be useful for diagnosing cerebral toxoplasmosis, particularly in patients with active disease. As shown here, there were no significant differences between the frequency and concentration of Toxoplasma-specific IgG1 and IgG2 antibodies in CSF samples from patients with cerebral toxoplasmosis based on an ELISA standardized with an antigen preparation from T. gondii cysts.

Protein antigens elicit mainly IgG1 and IgG2 antibodies, while in adults polysaccharide antigens preferentially induce antibodies of IgG3 and IgG4. The Toxoplasma tachyzoite-bradyzoite differentiation is associated with morphological and molecular changes, including the expression of stage-
specific proteins such as surface antigens or enzymes and alterations in parasite metabolism that are probably needed for parasite adaptation to environmental changes\textsuperscript{4,21}. Two major changes in the sugar content occur during cyst formation, namely, the synthesis of large amounts of polysaccharide that is stored as amylopectin granules in the bradyzoite cytoplasm and the presence of lectin-binding sugars in the cyst wall\textsuperscript{4,23}. The presence of polysaccharides (carbohydrates) in the cyst antigen preparation used in our ELISA could account for the high frequency of IgG\textsubscript{2} detected here.

Some studies have suggested that *Toxoplasma*-specific IgG subclasses may be markers of congenital and cerebral toxoplasmosis or clinical outcome\textsuperscript{2,3,5,23}. In a study using an ELISA standardized with tachyzoite antigens and serum samples from mother/newborn pairs with maternal exposure to *T. gondii*, CAÑEDO-SOLARES et al. (2008) showed that IgG\textsubscript{1} in the mothers and IgG\textsubscript{2} in the newborns were related to offspring clinical problems; IgG\textsubscript{1} and IgG\textsubscript{2} in babies were markers of vertical transmission, whereas IgG\textsubscript{1} in mothers or children was associated with clinical problems. DE SOUZA-E-SILVA et al. (2013), in a study designated to evaluate the association between clinical signs of congenital toxoplasmosis and IgG subclasses, showed that the detection of anti-paraMIC\textsubscript{3} IgG\textsubscript{2} and IgG\textsubscript{3} was associated with the presence of retinochoroidal lesions and intracranial calcifications. MEIRA et al. (2013) showed that the detection of IgG\textsubscript{2} specific for *T. gondii* IgA in serum and/or CSF supported the diagnosis of cerebral toxoplasmosis in HIV-infected patients.

Our results confirmed the predominance of IgG\textsubscript{1} antibodies in the immune response to *T. gondii*. However, IgG\textsubscript{1} antibodies were found at a frequency and concentration similar to IgG\textsubscript{2} antibodies in CSF samples from patients with cerebral toxoplasmosis. The marked IgG\textsubscript{2} response in CSF from patients with cerebral toxoplasmosis reported here merits further investigation.

**RESUMO**

Resposta de anticorpos específicos das subclasses da IgG para *Toxoplasma* em amostras de líquido cefalorraquidiano de pacientes com toxoplasmose cerebral

A toxoplasmose cerebral pode ser altamente debilitante e ocasionalmear fatal em pessoas com deficiências do sistema imune. Nesse estudo, nós avaliamos a resposta de anticorpos das subclasses da IgG para o Toxoplasma gondii em 19 amostras de líquido cefalorraquidiano (LCR) de pacientes com toxoplasmose cerebral que apresentavam uma reação IgG anti-*T. gondii* positiva com ELISA padronizada com uma preparação antígénica de cistos. Nós encontramos diferenças significativas entre as taxas de positividade e as concentrações de anticorpos (média aritmética das absorbâncias das reações ELISA, MEA) para IgG\textsubscript{1} e IgG\textsubscript{2}, mas as taxas de positividade e valores MEA para estas duas subclasses de IgG foram signficativamente superiores aos da IgG\textsubscript{3} e IgG\textsubscript{4}. A resposta marcante de anticorpos IgG\textsubscript{2} em LCR de pacientes com toxoplasmose cerebral merece investigação adicional.

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