Comparative Immunology, Microbiology and Infectious Diseases 38 (2015) 9–13

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Immunological response and markers of cell damage in seropositive horses for *Toxoplasma gondii*

Guilherme M. Do Carmo[a], Aleksandro S. Da Silva[b,*], Vanderlei Klauck[b], Rafael Pazinato[b], Anderson B. Moura[c], Thiago Duarte[d], Marta M.M.F. Duarte[e], Guilherme V. Bochi[d,f], Rafael N. Moresco[d,f], Lenita M. Stefani[b]

[a] *Postgraduate Program of Nanoscience, Centro Universitário Franciscano, Santa Maria, RS, Brazil*
[b] *Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil*
[c] *Department of Veterinary Medicine, UDESC, Lages, SC, Brazil*
[d] *Postgraduate Program in Pharmacology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil*
[e] *Universidade Luterana do Brasil, Santa Maria, RS, Brazil*
[f] *Research Laboratory of Clinical Biochemistry, Department of Clinical and Toxicological Analysis, UFSM, Santa Maria, RS, Brazil*

ARTICLE INFO

Article history:
Received 14 September 2014
Received in revised form 4 December 2014
Accepted 11 December 2014

Keywords:
Immunoglobulin
Cytokines
Horses
Toxoplasmosis

ABSTRACT

Toxoplasmosis is an important parasitic disease affecting several species of mammals, but little is known about this disease in horses. This study aimed to investigate the levels of several immunological variables and markers of cell damage in the serum of seropositive horses for *Toxoplasma gondii*. Sera samples of adult horses from the Santa Catarina State, Brazil used on a previous study were divided into groups according to their antibody levels for *T. gondii* determined by immunofluorescence assay, i.e. 20 samples from seronegative horses (Group A – control), 20 samples from horses with titers of 1:64 (Group B), 20 samples of horses with titers of 1:256 (Group C), and five samples from horses with titers of 1:1024 (Group D). Positive animals (Groups B, C, and D) had higher levels of immunoglobulins (IgM and IgG), pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1, IL-4, and IL-6) and protein C-reactive protein, as well as lower levels of IL-10 (anti-inflammatory cytokine) when compared to seronegative horses (Group A). The nitric oxide levels were also elevated in seropositive horses. Therefore, we have found humoral and cellular immune responses in seropositive horses, and a correlation between high antibody levels and inflammatory mediators. Markers of cell injury by lipid peroxidation (TBARS) and protein oxidation (AOPP) were elevated in animals seropositives for *T. gondii* when compared to seronegatives. Therefore, seropositive horses to *T. gondii* can keep active immune responses against the parasite. As a consequence with chronicity of disease, they show cellular lesions that may lead to tissue damage with the appearance of clinical disease.

1. Introduction

Toxoplasmosis is a protozoal disease widely distributed in the American continent and affects many warm-blooded vertebrates. This disease is caused by *Toxoplasma gondii*, an obligate intracellular parasite of the Sarcocystidae family [1,2]. Felidae are the definitive host and many vertebrate species can be intermediate host, both usually with asymptomatic disease [3]. When clinical signs occur, they may vary among the species involved resulting from a simple fever and mild lymph node enlargement to severe cases with nervous system involvement, blindness, and

* Corresponding author. Tel.: +55 04933309440.
E-mail address: aleksandro_ss@yahoo.com.br (A.S. Da Silva).

http://dx.doi.org/10.1016/j.cimid.2014.12.001
0147-9571/

abortions [4–6]. *T. gondii* infection is characterized by a marked cellular immune response [7], which may induce fever [8]. Although clinical disease caused by the parasite is rare, toxoplasmosis is often associated with cases of immunosuppression by being an opportunistic parasite [9].

The immunity against *T. gondii* is complex and involves two types of response: humoral and cellular [10]. The humoral response is characterized by the production of specific immunoglobulins (Ig) by B lymphocytes, mainly IgG and IgM. On the other hand, cellular immune response is mediated by T lymphocytes, which secrete cytokines, well known inflammatory mediators capable of killing extracellular tachyzoites [11].

Among domestic animals, small ruminants are the main ones showing clinical signs of toxoplasmosis, mainly related to reproductive problems [6]. In Brazil, around 32% of horses are seropositive to *T. gondii* [12,13], but the seroprevalence may vary from 0% up to 90% in natural conditions [14]. However, most of them are chronically infected and asymptomatic. According to the literature, clinical signs in horses are different ranging from incoordination, abortions, excessive irritability [15], to hyperthermia, lack of appetite, sluggishness, diarrhea, ocular mucous secretion, and nasal discharge [16]. In view of this, our hypothesis is that equine immune responses against *T. gondii* are lasting, variable and a contributor factor for the disease pathogenesis and cellular lesion. In this context, the aim of this study was to investigate immunological variables and markers of cell damage in the serum of seropositive horses for *T. gondii.*

2. Materials and methods

2.1. Serum samples

Sera samples stored at −80 °C of adult horses from the mountainous region (mountainous plateau) the Santa Catarina State, Brazil were used this study. The assessment of IgG anti-*Toxoplasma* was carried out by indirect immunofluorescence assay (IFA), according to the technique described by Camargo [17]. Briefly, the tachyzoites of *T. gondii* (RH strain) were used as antigens, obtained of peritoneal fluid containing parasites from experimentally infected mice. Anti-IgG antibodies of horse, conjugated with fluorescein were used as the secondary antibody in this reaction. A sample was considered as positive when fluorescence occurred on the entire tachyzoite surface and negative when fluorescence was absent, or only apical. Seropositive and negative samples were used as the controls. The sera were tested for antibodies at a dilution of 1:64, and positive samples were tested up to the maximum dilution, at which was possible to detect antibodies against the protozoa.

A total of 45 seropositive horses for *T. gondii* was used in this study, and negative samples for *Neospora* spp. and *Sarcocystis* spp. (analysis by serological tests). Seronegative samples for the three protozoa were used as negative control. Thus, the groups were formed as follows: 20 sera samples from not-infected horses (Group A – control), 20 of *T. gondii* infected horses (Group B – titers 1:64), 20 of *T. gondii* infected horses (Group C – titers 1:256), and 5 of *T. gondii* infected horses (Group D – titers 1:1024) in order to evaluate immunoglobulins, cytokines, C-reactive protein levels, concentration of nitrite/nitrate (NO_x), thiobarbituric acid reactive substances (TBARS), and advanced oxidation protein products (AOPP). The samples were processed in duplicate for all those described below. The samples were stored for about six months, and thawed only on the day of immunological and biochemical processing. Group D comprised only five animals, because they are natural, and hard to be identified cases. The other groups were formed by 20 animals, in order to have a meaningful sampling for the variables studied for naturally infected animal, as this natural situation the variation could be great.

2.2. Immunoglobulin levels

Serum IgG and IgM levels were determined using immunonephelometry on the Behring Nephelometer BN II (Dade Behring – USA) with reagents from Dade Behring, and specific kits for IgG (Horse IgG(T) ELISA Quantitation Set®, Bethyl Laboratories, USA), and IgM (Horse IgM ELISA®, Kamiya Biomedical Company, Seattle). Briefly, all samples were diluted into specific diluents and measured after 10 min according to technical instructions in kit. Polystyrene particles were coated with a specific monoclonal antibody for each serum protein, forming an agglutinate that disperses the light irradiated in the presence of the protein. The intensity of scattered light depends on the amount of protein concentration in the sample, and the results are compared with known standard curves [18].

2.3. Cytokines levels

Cytokine quantification (TNF-α, INF-γ, IL-1, IL-4, IL-6, and IL-10) was assessed by ELISA assay using commercial Quantikine immunoassay kits (GSI Equine – Plasma/Serum DataSheet, Genorise Scientific, Inc., USA) according to the manufacturer's instructions. Briefly, 96-well microplates were sensitized with primary antibody at room temperature for 30 min; the sample was added and incubated for 30 min at 37 °C. After washing, the secondary antibody conjugated with peroxidase was added to each well and incubated. The concentration of the cytokines was determined by the intensity of the color measured spectrophotometrically using a microplate reader.

2.4. C-reactive protein levels

The quantification of serum C-reactive protein (CRP) was performed using commercial kits of ultrasensitive CRP (BioTécnica, Minas Gerais, Brazil), following the manufacter's protocol at semiautomatic analyzer Bio-2000. The test samples were treated with a specific antibody for horse in a suitable buffer. The turbidity induced by the formation of immune complexes was measured at 540 nm, and the values were then calculated automatically when compared to known standards. All the assay steps were performed automatically by spectrometry (BioTécnica, Minas Gerais, Brazil).