SEROLOGICAL DIFFERENTIATION BETWEEN ACUTE AND CHRONIC STAGES IN EXPERIMENTAL *TOXOPLASMA* INFECTION OF NORMAL AND IMMUNOSUPRESSED RATS (*Rattus norvegicus*)

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SUMMARY

The immune response of toxoplasmosis can give good information to an accurate vigilance of food products. This study analyzed anti-*Toxoplasma gondii* antibodies kinetics, with or without immunosuppression. Three groups of four rats, G1 and G2 infected with bradyzoites, G2 immunosupressed, and G3 control, were evaluated weekly for modified agglutination test (MAT), with methanol (AC) or formalin (HS), and immunofluorescent antibody test (IFAT). IFAT did not differentiate acute and chronic stages. MAT-AC detected antibodies only from acute stage, while MAT-HS from both stages. Thus, the differentiation of the stages is possible from sequenced sera samples.

Keywords: *Toxoplasma gondii*, *Rattus norvegicus*, serology, experimental infection

INTRODUCTION

Toxoplasma gondii is an obligate parasite protozoan, worldwide distributed, that infects warm-blooded hosts, including human beings. It has a great importance to immunocompromised patients being an opportunistic parasite, causing serious sequels like toxoplasmic encephalitis, and to pregnants, causing fatal damages to fetus (Dubey & Beattie, 1988; Smith, 1997). The hosts can be infected for the consumption of raw and undercooked meat of a chronic infected animal containing cyst with bradyzoites, food or water contaminated with sporulated oocysts eliminated for infected cats in its faeces, or for transplacental transmission of tachyzoites (Tenter et al., 2000).

In immunocompromised patients, the reacutization of chronic infection occurs due the low cellular and humoral responses, releasing bradyzoites from tissue cysts that convert in tachyzoites, dissemining to all host tissues, i.e., brain, lung, heart, liver, spleen (Carruthers, 2002; Luft & Hafner, 1990).

Tachyzoites and bradyzoites of *T.gondii* have in them surfaces specific antigens. Modified agglutination test (MAT) allows detecting specific antibodies from these antigens of the acute and chronic stages, of any species, only dependent of antigen-antibody interaction. While this, methanol (MAT-AC) exposes specific antigens from acute stage (tachyzoites, 24KDa), not presenting crossed-reaction with bradyzoites, while formalin (MAT-HS) exposes both antigen

from acute and chronic stages, i.e., bradyzoites 35Kda antigen (Dubey & Beattie, 1988; Thulliez et al., 1986).

This study aimed to analyze experimentally the dynamic of antibodies and the applicability of serological tests for the diagnosis of toxoplasmosis, comparing the evolution of antibodies in infected rats, immunosupressed or not with corticoids, and measuring specific antibodies from each stage.

MATERIAL AND METHODS

All experimental study was realized in the laboratories of the Zoonosis Researches Nucleus, FMVZ, UNESP, Campus of Botucatu, SP.

Three experimental groups were used in this study. G1 and G2 groups, four Wistar rats (*Rattus norvegicus*) each groups, infected with 10^4 bradyzoites of BTU 10 strain, genotype I, p.o., being that G2 was immunosupressed with corticoid (Dexamethazone, p.o. + Hydrocortisone succinate, s.c.) since 155th day after infection, according to the protocol described for Djurkovic-Djakovic & Milenkovic (2001), adapted by interspecific allometric extrapolation protocol (Pachaly & Brito, 2001); G3, four Wistar rats inoculated with saline solution, as control.

The blood samples were collected weekly, during 90 days (G1 and G3), and 180 days (G2), by the punction of the orbital sinus. The samples were centrifuged at 1600g per 10 minutes, and the sera samples were frozen until the moment of the test.

The serological tests used were the modified agglutination test (MAT) and immunofluorescent antibody test (IFAT).

MAT-HS (Desmonts & Remington, 1980) using formalin, and MAT-AC (Silva, 2006), using methanol to fix the antigens, were realized to measure titres of antibodies from both stages, and to analyze the reacutization of the infection. A clear-cut button-shaped deposit of parasite suspension at the bottom of the well was considered negative, and a complete carpet of agglutinated organisms was positive (Figure 1).

IFAT (Camargo, 1974) was realized using FITC anti-rat-IgG and FITC anti-rat-IgM (Bethyl laboratories ®), according to the instruction of the manufactory, in a Zeiss SH250 fluorescence microscope, 40x. An intense fluorescence in all membrane of tachyzoites in 50% or more, per field, was considered positive to this dilution, until the final dilution of the test.

All sera samples were evaluated to MATs and IFAT. The titres of antibodies were converted in log (10*titre), and the area under the curve of the titres (AUC) obtained (Jungersen et al., 2002). AUC of different serological tests were compared by Student t Test, to compare two tests, or by One Way Analysis of Variance, with comparison of averages by Tukey test. The comparison of the AUC of the intervals of weeks for each one test was realized for the Analysis of Variance for Paired Groups, with comparison by Tukey test.

RESULTS AND DISCUSSION

To both groups, G1 and G2, IgM titres were identified already since the first days, with higher levels than IgG until 14 days of infection, where IgG starts the production and display equal or higher levels than IgM between the 3rd and 4th weeks. Naot & Remington (1980) verified the same, but comparing ELISA-IgM and IFAT-IgM, where basal level of IgG is produced, when a peak of IgM is present.

Between the 2nd and 3rd weeks, IgM and IgG display same levels. This occurs because the immune response of the host was researching of a better form to combat the infection, and with IgM, in the first days is essential due the rapid production but with low specificity. With the progressive maturation of the infection, a specific immune response is produced, mainly with IgG.

After the 3rd and progressive weeks, a same response of antibodies was observed in both groups. IgM and IgG maintained the same level with a discrete and progressive decline of IgM, which is observed with IgG too, but with minor intensity. In G2, important data could be observed in relation to the differentiation of antibodies. IgM and IgG could not be differentiated, in rats, after the 3rd week, along of 23 weeks studied.

To IFAT is important to consider that, in rats, IgM can give an important indication of acute infection. But we should to consider too that the immune response in toxoplasmosis, is very specify, and *T.gondii* epitopes are very immunogenic. So that, the immune response is very intense, maintaining higher titres of antibodies for a long time. Thus, higher titres of IgM and IgG can be observed for many weeks, as observed in this study. With this, and observing the Graphic 1, the differentiation between acute and chronic stages, based in IgM and IgG, in rats, is very difficult after the first weeks.

Evaluating MATs at the final of the 2nd week, IgG starts to be detected, and until the same week only IgG from acute stage (IgG-AC) was detectable. Between the 2nd and 3rd weeks, IgG-AC presented lower levels than total IgG detectable, being this final IgG the IgG-AC added of those from chronic stages, which was in lower quantity in the studied period, agreeing with Thulliez et al. (1986).

Along the 13th weeks, the differentiation increased characterizing that the maturation of the immune response starts early, in minor intensity, and is occurring, advancing for a long time. This fact can be better observed in G2, along of 23 weeks. However, MAT allows the differentiation between acute and chronic stages, as studied for Dannemann et al. (1990).

A peak of antibodies was identified between the 3rd and 4th weeks. After this, IgG levels reduced gradually, most emphasized in those from acute stage, which demonstrate the maturation gradually of the immune response.

Both MAT-AC and MAT-HS presented significant difference ($P<0.05$) between both groups, G1 and G2. MAT-HS differed significantly to IFAT-IgG and to IFAT-IgM ($P<0.05$). In contrast, both IFAT tests not differed significantly each other ($P>0.05$), as well as MAT-AC and IFAT-IgG to G1, which occurred to G2 ($P<0.05$). All animals of G3 were negative serologically.

After the immunosupression, in G2, at 23rd week was observed an increase of IgG, to IFAT-IgG as well as MATs, but most emphasized in MAT-HS. Curiously, after the introduction of immunosupression, in IFAT-IgG was observed a rapid decline, followed for an increase of antibodies. This fact could be explained for rapid sensitization of the immune system, followed for a recuperation and elevation in antibodies levels at the immune response. Djurkovi-Djakovic & Milenkovic (2001) verified that the prolonged use of corticoid may cause considerable toxicity, with progressive loss of weight and death.

In the Table 1, only IFAT-IgG differentiate significantly the last period, 22nd to 26th week, to the 4th period, 17th to 21st week, which deserve other studies to characterize this reacutization, because only this test had detected, statistically, the immunosupression serologically.

To differentiate the stages of *T.gondii* infection and to contribute effectively to public health (Desmonts & Remington, 1980; Marca et al., 1996), a period upper than six months should be, with paired sera samples considered, allowing the characterizing of the behavior of the antibodies anti-*T.gondii* along the infection.

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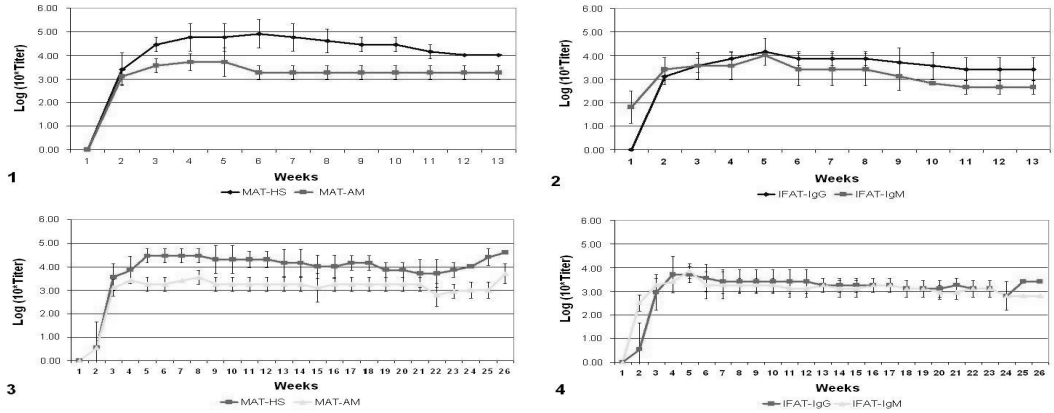

Graphic 1. Week variation of the concentration of anti – *T.gondii* antibodies in immunosupressed or not experimentally infected rats. Botucatu, 2006

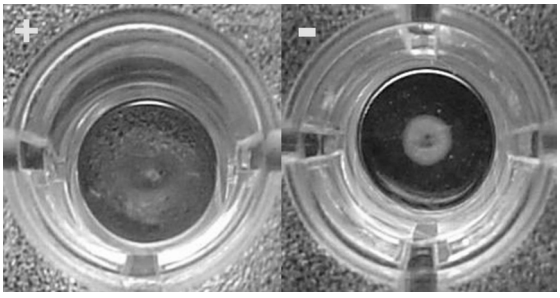

Figure 1. Positive (left) and negative (right) microscopic evaluation for MAT. Botucatu, 2006

Table 1. Comparison among the means of curves of the concentration of antibodies, in different intervals, in animals submitted to immunosupression, according to the serological test used. Botucatu. 2006

Test	Periods / Mean ± Standard deviation of the area of the curve					F Statistics	P Value
	1st a 6th week	7th a 11th week	12th a 16th week	17th a 21st week	22nd a 26th week		
MAT-HS	$2.44^{A} \pm 0.34$	$3.49^{B} \pm 0.35$	$3.30^{B} \pm 0.39$	$3.16^{AB} \pm 0.23$	$2.88^{AB} \pm 0.59$	1.7140	0.0146
MAT-AC	$1.99^{A} \pm 0.26$	$2.68^{B} \pm 0.18$	$2.58^{AB} \pm 0.30$	$2.61^{AB} \pm 0.24$	$2.15^{AB} \pm 0.51$	1.4160	0.0156
IFAT-IgG	$2.12^{A} \pm 0.45$	$2.73^{AB} \pm 0.39$	$2.62^{AB} \pm 0.25$	$3.15^{B} \pm 0.28$	$2.22^{A} \pm 0.55$	4.1360	0.0038
IFAT-IgM	$2.44^{A} \pm 0.13$	$2.59^{A} \pm 0.24$	$2.53^{A} \pm 0.24$	$2.46^{A} \pm 0.21$	$2.12^{A} \pm 0.31$	1.7830	0.0719

Statistical results: values of P less than 0.05 indicate significant difference among the curves of concentration of antibodies in the studied periods, for Analysis of Variance to dependent samples – value of averages followed by different words indicate significant differences among the periods, to one test of detection of antibodies, considering a significance level of 5%.