Impact of Spiramycin Treatment and Gestational Age on Maturation of *Toxoplasma* IgG Avidity in Pregnant Women

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ABSTRACT

The objective was to investigate the maturation of IgG avidity after *Toxoplasma* seroconversion during pregnancy and the factors that affect IgG avidity over time. The study used 309 sera from 117 women and a multiple linear mixed regression to show the pattern of variation of IgG avidity along gestation.

The IgG-avidity ratios and the patterns of their evolution with time were quite diverse among women and statistically heterogeneous (test, \( p=0.011 \)); however, the trend was towards a statistically significant increase (test, \( p<0.0001 \)). In average, a 1.0167-fold increase was observed for each additional gestational week after the putative date of infection. At 12 weeks after putative infection (the expected IgG-avidity maturation time), the mean avidity ratio was 16.6% (95% confidence interval, 15.4–17.9). At all times, the avidity ratio remained significantly heterogeneous among women (\( p<0.05 \)); for 95% of them, that ratio ranged from 7.8 to 35.3% at 12 weeks after putative infection. Maternal age at putative time of infection did not influence maturation of IgG avidity. However, in average, a 1.009-fold decrease (\( p=0.03 \)) in that avidity was observed for each additional week of gestational age before infection and a 1.03-fold increase (\( p=0.0003 \)) for each additional week of delay to onset of spiramycin treatment. The rate of increase in avidity ratio was lower as infection occurred late in pregnancy, higher as the delay to treatment was long.

This information cannot allow accurate determination of the delay since infection. The present results provide support for interpretation of the assay and caution against over interpretation.

**Key words:** Toxoplasmosis, IgG avidity, Treatment, Gestational age, Heterogeneity.
INTRODUCTION

Acute Toxoplasma infection can have serious consequences on fetal development including abortion, neurological and ocular fetal lesions, and subclinical infections (26). Moreover, some toxoplasma-linked ocular lesions appear or relapse during childhood or adolescence (25, 33). This is why, in France, a monthly serological follow-up during pregnancy was made mandatory for seronegative women since 1992 (32).

The risk of mother-to-child transmission of Toxoplasma gondii was shown to be inversely proportional to the stage of pregnancy at which maternal infection occurs (9, 17). Thus, dating maternal toxoplasma infection during pregnancy is of great importance because it allows determination of the fetal risk. Today, various serological markers may be used to estimate the time elapsed since Toxoplasma seroconversion, including the measurement of immunoglobulin G (IgG) avidity (16). Although it is well recognized that a high IgG-avidity ratio can rule out acute infection, a low IgG-avidity ratio is insufficient to infer a recent infection (21). Moreover, data on the evolution of IgG-avidity ratio over time after Toxoplasma infection are lacking. Though a heterogeneity of that evolution has been already reported (13), studies on the effect of treatment on that evolution have reported contradictory findings (13, 18, 24, 28), and studies on the effects of other factors are still awaited for.

The aim of this study was to investigate the patterns of maturation of IgG avidity after Toxoplasma seroconversion during pregnancy and to determine factors that could influence the evolution of IgG-avidity ratio.
MATERIALS AND METHODS

Pregnant women inclusion criteria

The evolution of IgG-avidity ratio over time was studied in a retrospective cohort of pregnant women in whom *Toxoplasma* seroconversion was confirmed in our laboratory between April 2001 and November 2003.

For each woman, the time of conception was obtained from the medical records. In this cohort, in accordance with law, all women had monthly follow-up visits. Thus, the putative date of infection was first determined between the last negative and the first positive IgG test. Then, a set of other clinical and serological criteria (IgM and IgG kinetics) allowed determining the putative date of infection to an accuracy of within a week (3, 20, 30).

Data on monthly IgG titers from seroconversion till the end of pregnancy were extracted from our laboratory database. Pregnant women with too low IgG titers to determine IgG-avidity ratio were excluded from the analysis.

All pregnant women who seroconverted were treated with spiramycin (9 MUI/day) until the end of pregnancy. The delay between *Toxoplasma* seroconversion and the onset of spiramycin treatment was calculated. Women who received treatments other than spiramycin were excluded.

Finally, the set of data kept for analysis concerned 117 pregnant women and 309 avidity ratio values. Each pregnant woman participated one to seven consecutive serum samples (mean 2.6 and median 2 samples per woman). These sera were drawn 1.3 to 41 weeks after putative infection, half of them within 9.6 weeks. Women follow-ups ranged from 3 to 41.6 weeks; half of the follow-ups were longer than 13.7 weeks.
Avidity assay

Toxoplasma specific IgG was detected with Enzygnost® Toxoplasmosis/IgG enzyme-linked immunosorbent assay (ELISA) kit (Dade Behring, Marburg, Germany). After appropriate dilution, samples were divided into two aliquots that were incubated then washed: one with the wash solution of the kit and the other with a homemade wash solution with 6 M urea. This was followed by washing, labelling with peroxidase-conjugated anti-human IgG, addition of substrate for revelation, A450 reading, and calculation of IgG titers (For more details, see Ref. 3). The percent IgG-avidity ratio was the ratio of the absorbances with and without urea treatment x 100. This value will be referred to later as the "IgG-avidity ratio" or "avidity ratio", according to the context. A cut-off for high avidity ratio was set at 38% to exclude infections acquired 12 weeks or less before sampling, 12 weeks being the theoretical time of IgG-avidity maturation.

The reproducibility of the avidity ratio was estimated by replicate measurements on two known sera with low and high avidity ratios. The coefficients of variation of intra-assay reproducibility (n = 40) were 10.8% and 15.0%, respectively and the coefficients of variation of inter-assay reproducibility (n = 30) were 10.4% and 12.8%, respectively.

Factors assumed to influence maturation of IgG avidity

Various factors (covariates) likely to influence the maturation of IgG avidity over time were analyzed: gestational age at the putative time of infection, delay to spiramycin treatment after the estimated date of infection, and maternal age at putative time of infection.
Statistical analysis

Distribution of the IgG-avidity ratios

The histogram that represented the distribution of the original percent IgG-avidity ratios was found skewed to the left. After a neperian logarithmic transformation, the distribution of the log-transformed avidity ratios, noted ln(IgG-avidity ratio), showed a close to normal distribution. This step was needed for the subsequent modelisation step.

Modelisation

A multiple linear mixed regression (14) was used to modelize the evolution of the percent IgG-avidity ratios after Toxoplasma seroconversion. The ln(IgG-avidity ratio) was used as dependant variable.

The mixed regression model, including both fixed and random effects, allowed to modelize individual evolutions of the avidity ratio and to quantify and explain heterogeneity among women. The random effects on the intercept and the slope of the regression line were used to quantify respectively the heterogeneity of these two parameters around the mean ln(IgG-avidity ratio) at any given time and around the mean rate of increase of this ratio along time.

The factors assumed to influence maturation of IgG-avidity were introduced in the model as fixed effects to explain heterogeneity.

In all tests, the level of statistical significance was set at $p=0.05$. All analyses were performed using the R 2.0.1 software (Free Software Foundation, Boston, MA, USA).
RESULTS

Evolution of the IgG-avidity ratio along time

Plotting the original percent IgG-avidity ratios versus time after putative infection showed that the original ratios and the patterns of evolution were quite diverse among women (Fig. 1a). Plotting model-predicted evolution using the log-transformed avidity ratios showed lines of different slopes ranging from -0.017 to +0.051 for 95% of them. These slopes were significantly heterogeneous among women (p=0.011). However, the general evolution trend was that of an increase in avidity ratios along time (Fig. 1b). That increase was statistically significant (p<0.0001). In average, an increase of 0.0167 in the log-transformed ratios was observed for each additional week after putative infection, which corresponds to a 1.017-fold increase with the original percent IgG-avidity ratio values.

Besides, with this model, a mean avidity ratio can be determined at any given time after putative infection. For example, 12 weeks after putative infection --the expected maturation time for IgG avidity-- the mean avidity ratio was 2.811 (95% confidence interval, CI, 2.735–2.887) with the log-transformed values, which corresponds to 16.6% (95% CI, 15.4–17.9) with the original values. However, the avidity ratio remained significantly heterogeneous among women (p<0.05). For example, at 12 weeks, for 95% of them, that ratio ranged from 2.059 to 3.563 with the log-transformed values; i.e., from 7.8 to 35.3% with the original ones.

Factors that influence maturation of IgG avidity

The influence of gestational age at putative infection on the IgG-avidity ratio was significant (p=0.03). In average, the ln(IgG-avidity ratio) decreased by 0.009 for each additional week of pregnancy before infection, which corresponds to a 1.009-fold decrease with the original percent IgG-avidity ratios (See Fig. 2a for three selected gestational ages at infection).
The influence of the delay to spiramycin treatment on the avidity ratio was also significant ($p=0.0003$). In average, the ln(IgG-avidity ratio) increased by 0.03 for each additional week of delay to the onset of spiramycin treatment, which corresponds to a 1.03-fold increase with the original percent IgG-avidity ratios (See Fig.2b for three selected delays to treatment).

Besides, working with the log-transformed IgG-avidity ratios, we found that rate of increase in the avidity ratio fell by 0.0003 for each additional week of pregnancy at the time of putative infection but raised by 0.0009 for each additional week of delay to treatment (See Fig. 2a and 2b respectively). However, these trends were not statistically significant.

Interestingly, even after adjustment on gestational age at putative infection and on the delay to treatment, the percent avidity ratio remained heterogeneous among women. For example, at 12 weeks after putative infection it varied from 9 to 30% for 95% of the women.

The mean age of the pregnant women at the putative time of seroconversion was 31 years (SD: 9.9 years; range: 19–43 years). Maternal age at putative time of infection was not found to influence the maturation of IgG avidity (data not shown).
DISCUSSION

We showed that the effect of gestational age on IgG-avidity ratio was significant and independent from the delay to spiramycin treatment; the avidity ratio was all the more low as maternal infection occurred later in pregnancy and as the delay to spiramycin treatment was short.

The significant increase in IgG-avidity ratio over time we found completes previous results on: i) the evolution of immunoglobulin affinity during immune response (11); ii) the persistence of low IgG-avidity ratios even 11 years after infection in some immunocompetent patients and pregnant women (21); and iii) the slow increase over two years of IgG avidity in congenitally infected children (2). Though we report here a constant increase in IgG-avidity ratio with time after putative infection, this increase was so heterogeneous between women that it could not be used for accurate determination of the time elapsed since infection. This heterogeneity was partially explained by two factors: gestational age at the putative time of infection and the delay to the onset of treatment. However, even when these two factors were taken into account, a great heterogeneity persisted. Besides, we found that maternal age is not likely to be involved. The reproducibility of our tests was also sufficiently high to exclude technical problems. According to reports on experimental infections, maturation of IgG avidity may be affected by some factors related to the parasite, such as the size of the inoculum (12) or the parasitic stage at infection (7). In human infection, such factors cannot be monitored. Host-related factors, such as dendritic cell response (8) or genetic susceptibility to infection (31), could also interfere with the maturation of IgG avidity but this issue was not addressed in this study.

The effect of gestational age at seroconversion on the maturation of IgG avidity has not been reported previously. This could be explained by the fact that T-cell immune response is modified during pregnancy to avoid rejection of the fetus (34). The role of CD4+ CD25+
regulatory T cells during pregnancy has been recently outlined (35). These cells seem to control the production and maturation of affinity of antigen-specific antibodies (10); they proliferate during the second and the third trimesters of pregnancy (29). This could partially explain the delay to maturation of *Toxoplasma*-specific avidity until the late phase of pregnancy. In addition, some Th3 cytokines (transforming growth factor β) produced by the placenta during the third trimester of pregnancy could decrease the maturation of IgG avidity (19).

Studies on the influence of treatment on the maturation of IgG avidity have shown conflicting results. Our results agree with those of Sensini *et al.* (28) and Petersen *et al.* (24). Sensini *et al.* (28) first hypothesized that treatment slowed down maturation of avidity and compared the mean IgG avidity values between treated and untreated women but they did not specify the type of treatment or its length. In two other studies, treatment was reported not to influence the maturation of avidity. However, Jenum *et al.* (18) compared the mean IgG avidities of 85 sera before and after treatment of 23 pregnant women with *Toxoplasma* seroconversion but they did not inform on the time interval between the two drawings or on the type or length of treatment. Flori *et al.* (13) compared IgG avidity in four groups with different treatment lengths and a control one without finding significant differences. Being dependant on gestational age at infection and highly correlated with the putative time of infection, the length of spiramycin treatment cannot be taken into account in the regression analysis. In fact, the regression analysis did not consider the length of treatment but the delay to treatment which is not related to the gestational age.

Although not well-understood yet, a role of treatment, especially spiramycin, on the maturation of IgG avidity is highly probable. The action of macrolides on *Toxoplasma gondii* has not been elucidated yet but they possibly act through inhibition of protein synthesis (1). Maturation of avidity is the result of different mechanisms such as somatic hypermutation of
rearranged Ig genes and the selection of B cells with high-avidity surface Ig by deletion of low-avidity B cells (23). Somatic hypermutation is a T cell-dependent antigen-driven immune response that occurs in the germinal centers of secondary lymphoid tissues. As high-affinity B cells require small amounts of antigen to be selected and to survive (15), it is conceivable that spiramycin delays maturation of avidity by reducing the amounts of *Toxoplasma* antigen that select high-affinity B cells (4, 5, 6). Another hypothesis is that spiramycin acts on the immune maturation of lymphocytes and secretion of cytokines without any direct effect on *Toxoplasma* (22, 27).

In conclusion, we have demonstrated that maturation of *Toxoplasma* IgG avidity in pregnant women is highly variable and that its evolution over time is influenced by gestational age at maternal infection and by the delay to the onset of treatment. Such variability should be kept in mind when interpreting toxoplasma serology in pregnant women. Further studies are needed to determine others factors that could influence maturation of IgG avidity and whether the kinetics of maturation of avidity could influence transmission of the parasite from the mother to the child or play a role in the severity of congenital toxoplasmosis. In fine, our attempt to bring to light a reliable link between IgG-avidity ratio and time since infection in toxoplasmosis was largely negative. Indeed, though avidity ratio generally increases during the follow up period, its heterogeneity between individuals is such that it cannot be used to accurately date toxoplasma infection. The present results do not indicate any additional usefulness of IgG avidity testing but they provide support for interpretation of the assay and caution against over interpretation.
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REFERENCES


LEGEND TO FIGURES

Figure 1 - Consecutive measurements of the percent IgG-avidity ratios along pregnancy vs. putative time after *Toxoplasma* infection in 117 pregnant women (309 sera) (1a) and predictions of the multiple linear mixed regression model after logarithmic transformation of the IgG avidity ratios (2b).

Figure 2 – Predicted increase of the ln(IgG-avidity ratio) with time after infection for three gestational ages at putative infection, the delay to treatment being fixed (1a) and predicted increase of the ln(IgG-avidity ratio) with time after infection for three delays of treatment, the gestational age at infection being fixed (2b).