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IgG anti-(Fab')2 Antibodies in Early Pregnancy Sera of Women with Anti-thyroid Antibodies and Normal Outcome or Spontaneous Abortions

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Abstract. *Anti-thyroid antibodies (atbs) (anti-TPO, anti-TG, anti-TRH receptor) are autoatbs that recognize specific antigens that belong to thyroid structures. Several mechanisms were proposed to explain the breaking of immunotolerance to thyroid antigens. Our previous studies showed that IgG-anti-F(ab')2 atbs exert immunosuppresives effect in vitro and inverse correlate with autoatbs in certain autoimmune diseases. Relying on sera from pregnant women with and without anti-thyroid atbs, respectively with or without spontaneous pregnancy loss we intended to analyze the IgG-anti-F(ab)2 atbs titers in sera of these categories of patients. The lot of patients consists of 126 pregnant women out of which 47 had a normal course of pregnancy and 79 experienced spontaneous abortion. Anti-TPO, anti-TG, and IgG-anti-F(ab')2 atbs titers were measured in sera of these women. Although a difference was found between IgG-anti-F(ab')2 atbs titer in pregnant women with positive versus negative anti-thyroid atbs, this was not statistically significant. IgG-anti-F(ab)2 atbs titer is higher in women with a normal course of pregnancy compared to women spontaneous pregnancy loss. Differently from other autoimmune diseases, our data show that IgG-anti-F(ab')2 atbs are not inversely correlated to anti-thyroid atbs titers. Higher IgG-anti-(Fab')2 atbs titers were found in pregnant women with normal course of pregnancy compared with those with pregnancy loss.*

Keywords: *autoimmune thyroiditis, pregnancy, IgG-anti-Fab2, immunotolerance*

1.Introduction

Anti-thyroid antibodies (atbs) are autoatbs that recognize antigens that belong to thyoid gland structures [1,2]. According to recognized antigenic structures, the atbs are stratified in anti-thyreoperoxidase (anti-TPO), anti-thyrogobulin (anti-Tg) and anti-TSH Receptor (TRAtbs) atbs [3]. The prevalence of these atbs in general population varies from 10-15% for anti-TPO to 3-6% for anti-TG and 1% for Trabs [1,4]. The most frequent consequences of the presence of anti-thyroid atbs are autoimmune thyroiditis and hypothyroidia, but in pregnancy the intrauterine milieu caused by the presence of these

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atbs could lead to impairment of various functions in the future child cardiometabolic syndrome, thyroid disease, IQ deficiencies [4,5,6], or pregnancy complications [7]. Other diseases found to be associated with anti-thyroid atbs are breast cancer [8], helicobacter pylori infections [9], systemic sclerosis [10], hyperprolactinemia [11], etc.

IgG-anti-Fab'2 atbs are part of an immunosuppressive mechanism [12]. Previous research showed that IgG-anti-Fab2 appear in sera of persons after immunization. Moreover, in vitro IgG-anti-Fab2 atbs suppress in vitro anti-erythrocyte atbs synthesizing autoimmune B cells [12]. Studies showed that in patients with systemic lupus erythematosus [13] and autoimmune hemolytic anemia, IgG-anti-F(ab')2 atbs titer inversely correlates with autoimmune antibodies titers [14,15]. Also transplant studies showed that increased suppressive IgG-anti(fab)2 atbs concentrations prolong graft surviving in transplant models and kidney graft recipients [16]. The specificity and chemical structures of antigens recognized by IgG-anti-Fab2 atbs were explained elsewhere [17].

Interestingly, although there are mechanisms that eliminate or suppress autoimmune cells, autoimmune atbs producing lymphocytes exist in the periphery of healthy people [13-15]. Such is the situation in autoimmune diseases. Pregnancy represents a particular period in the life of a woman when the fetus which is similar to a hemi allograft is not rejected [18]. The concomitant presence of an autoimmune disease and early pregnancy gave us the possibility to analyze the behavior of IgG-anti(Fab')2 atbs in this immunologically complex situation.

The scope of our research was to analyze the titer of IgG-anti-Fab2 atbs in sera of women with early pregnancy with and without pregnancy loss and in the presence of anti-thyroid atbs.

2.Material and methods

Population study

The lot of patient consists of 126 pregnant women out of which 47 had a normal course of pregnancy and 79 experienced a spontaneous abortion. Standard data were collected from the patients about medical anamnesis and other particualrities.

Collection of sera

Blood was collected by venipuncture on a procoagulant vacutainer. After separation blood was centrifuged at 500g for 10 minutes and sera were separated. The sera were isolated and frozen at -80 C until evaluation.

Determination of anti-TPO and anti-TG atbs

We used to test the sera an ELISA reader SUNRISE Remote, courtesy of Tecan GmbH, Austria. We used kits from DiaMetra (Spello, Italy) to measure the titer of anti-TPO (DK 0116) and anti-Tg (DK 0115) antibodies. The cut–off values were 20 (anti-TPO) and 5 (anti-TG). We presented elsewhere the detailed protocol. [19-20]

Measurement of IgG-anti-Fab2 antibodies titer

We coated ELISA plates with 1 pg/well of human F(ab')2 fragments (Jackson Immunoresearch Lab, West Grove, PA). We blocked remaining active groups with phosphate-buffered saline (PBS) + 1% gelatin. The test serum was diluted 1:l00 and added to prepared plates (50 uL/well, three wells for each sample). We used a positive control - a serum with a certain anti-F(ab')2 value. We used phosphate-buffered saline as negative control. In the next step we added alkaline phosphatase-conjugated goat anti-human IgG(Fc) antibody (Jackson Immunoresearch) 50 uL/well. We wash three times with PBS + 0.05 % Tween after each step. We added Substrate (250 ug p-nitrophenyl phosphate disodium/well) (Sigma Chemical CO, St Louis, MO). We measured the extinction (405 nm) every minute up to 90 minute. We stopped the measurement when the extinction (OD) of positive control reached a value of 1.2.

Statistical evaluation

We expressed values in median +/- Standard error of mean (SEM). We use the Mann-Whitney U rank sum test to compare values. We use Spearman rank corrected for ties to evaluate correlations. We use for statistical analysis GraphPad InStat software, San Diego, California, USA and SPSS, IBM Inc.

Ethical issues

The Committee of the University of Medicine and Pharmacy Timisoara approved our study (848/2011). We obtained consent from each patient.

3.Results and discussions

Many hypotheses were formulated to explain the breaking of tolerance to thyroid antigens and the synthesis of anti-thyroid antibodies which lead to autoimmune thyroiditis [21]. Since our working group studied an immunosuppresive mechanism represented by IgG-anti-F(ab')2 antibodies [17] which occurs in healthy people and patients, we aimed herein to analyze if these antibodies could play a role in the pathogenesis of synthesis of anti-thyroid autoantibodies.

Values of IgG-anti-F(ab')2 atbs titer in pregnant women with positive versus negative anti-thyroid atbs

If all pregnant women were included (pregnant women with normal pregnancy outcome and spontaneous abortion) those with positive anti-thyroid atbs (n=18) had higher IgG-anti-F(ab')2 values than those with negative anti-thyroid atbs titer (n=108): 0.78±0.14 vs. 0.64±0.06, p=0.84. However the difference did not reach a significant value. A similar trend was found if only women with normal pregnancy course [anti-TH+ (6) vs anti-TH- (41): 1.02±0.27 vs. 0.94±0.12, p=0.42) or with spontaneous abortion [anti-TH+ (12) vs anti-TH- (67): 0.72±0.17 vs. 0.62±0.07, p=0.85)] were analyzed.

Table 1. Comparison of IgG-anti-F(ab') 2 antibodies titer values in patients with positive or negative anti-thyroid antibodies: pregnant women with normal pregnancy outcome and spontaneous abortion patients

IgG-anti-F(ab')2 titer (OD)	Anti-thyroid atbs positive	Anti-thyroid atbs negative	P value
All	0.78±0.14 n=18	0.64±0.06 n=108	p=0.84
Normal pregnancy outcome	1.02±0.27 n=6	0.94±0.12 n=41	p=0.42
Spontaneous abortion	0.72±0.17 n=12	0.62±0.07 n=67	p=0.85

IgG-anti-F(ab')2 values (OD) are expressed in median± SEM

IgG-anti-F(ab')2 antibodies are antibodies which are synthesized along immunization and it is claimed that these antibodies have the role to limit the immune answer [17]. Previous studies confirm the immunosuppresive effect of IgG-anti-F(ab)2 antibodies on anti-erythrocyte antibodies producing B-lymphocytes cultures [22]. Moreover, studies showed that a lack of suppressive IgG-anti-F(ab)2 antibodies titers is associated with a relapse of autoimmune disease in lupus erythematosus and autoimmune haemolytic anemia patients [13-15]. Interestingly, our data do not show lower IgG-anti-F(ab')2 antibodies titer in patients with positive anti-thyroid antibodies compared with patients without positive anti-thyroid antibodies. Instead, contrary to expectation, we observed higher anti-F(ab')2 antibodies titer in patients with positive anti-thyroid antibodies titers. However, the difference did not reach a significant threshold. We have no explanation for these results but it may be possible that the IgG-anti-F(ab')2 antibodies are not involved in the pathogenesis of autoimmune thyroiditis. As described, it seems that many other mechanisms may be involved in the loss of tolerance to thyroid antigens such as deficit of central tolerance mechanisms, Treg, or structure of thyroid autoantigens [21].

IgG-anti-F(ab)2 atbs titer is higher in women with a normal course of pregnancy compared to women who experienced a spontaneous abortion

Our results showed that IgG-anti-F(ab')2 atbs titer is higher in early pregnancy sera of women with a normal course of pregnancy (n=47) compared to those with spontaneous abortion (n=79) (0.94±0.11 vs. 0.62±0.07, p=0.05). Interestingly, this difference was present no matter if patients with negative [NCP (41) vs. SA (67): 0.94±0.12 vs. 0.62±0.07, p=0.13)] or positive [NCP (6) vs. SA (12): 1.02±0.27 vs. 0.72±0.17, p=0.33)] anti-thyroid atbs titers were analyzed. In the last two groups the difference did not reach a significant threshold probably because of the small number of patients.

Table 2. Comparison of IgG-anti-F(ab')2 antibodies values in pregnant women with normal pregnancy outcome or spontaneous abortion with or without anti-thyroid antibodies

IgG-anti-F(ab')2 titer (OD)	Normal pregnancy outcome	Spontaneous abortion	P value
All pregnant women n=126	0.94±0.11 n=47	0.62±0.07 n-79	p=0.05
Anti-thyroid atbs negative n=108	0.94±0.12 n=41	0.62±0.7 n=67	p=0.13
Anti-thyroid atbs positive n=18	1.022±0.22 n=6	0.72±0.17 n=12	p=0.33

IgG-anti-F(ab')2 values (OD) are expressed in median± SEM

Our research gave us the possibility to analyze the behavior of the concentration of first trimester IgG-anti-F(ab')2 antibodies titers in sera of pregnant women with birth at term and spontaneous abortion. Interestingly, we found that the IgG-anti-F(ab)2 antibodies titer was higher in pregnant women with a normal course of pregnancy compared with those with spontaneous abortion. The difference was present in all groups of pregnant women stratified according to the presence of anti-thyroid antibodies but did not acquire significance.

Further studies should be performed on better characterized groups of patients to evaluate the course of IgG-anti-F(ab')2 antibodies along pregnancy and their role in spontaneous abortion or other pregnancy complications. Considering the risks to the fetus in pregnant women with anti-thyroide antibodies [4-7], we recommend performing the screening for thyroid pathology together with the other screenings [23-25].

4.Conclusions

Our study shows that unlike other autoimmune diseases such as autoimmune haemolytic anemia or lupus erythematosus where IgG-anti-F(ab)2 antibodies inversely correlate with autoantibodies titers, in our group of patients such a correlation did not occur. Interestingly, we found higher IgG-anti-(Fab')2 antibodies titer in pregnant women with normal course of pregnancy compared to those with spontaneous abortion. Studies with better characterized patients should be performed to analyze the course of IgG-anti-F(ab)2 antibodies titer in pregnant women.

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