

Antibodies against β_2 -glycoprotein I (β_2 -GP I) and Their Relationship to Fetoplacental Antigens

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Summary

Binding of β_2 -GP I to anionic phospholipids is thought to be the major antigen required in the reaction of anticardiolipin antibodies to phospholipids. The aim of this study was to investigate the changes of anti- β_2 -GP I IgG during the first and second trimester of pregnancy and the relationship between the levels of anti- β_2 -GP I and fetoplacental antigens and the correlation between anti- β_2 -GP I IgG and antibodies against oxidized low-density lipoprotein IgG (oLAb) in serum of pregnant women. We determined anticardiolipin antibodies (ACA) IgG and maternal serum levels of α_1 -fetoprotein (AFP), human chorionic gonadotrophin (HCG) and trophoblast-specific β_1 -glycoprotein (SP1) in 204 pregnant women in the first and second trimester. From this group we selected 52 serum samples positive for ACA IgG and 16 samples negative for ACA IgG. In the samples of selected patients, the levels of anti- β_2 -GP I IgG and oLAb IgG were determined. Anti- β_2 -GP I IgG levels significantly decreased in the second trimester (6.2 ± 9.3 U/ml, mean \pm S.D.) in comparison with the first trimester (8.3 ± 10.4 U/ml) ($p=0.05$). Multiple of median (MoM) AFP correlated negatively but not significantly in the first trimester with anti- β_2 -GP I ($r = -0.261$, $p = 0.12$). In the second trimester this correlation was significantly negative ($r = -0.278$, $p = 0.04$). The Spearman correlation coefficients for MoM HCG and anti- β_2 -GP I were 0.158 for the first trimester and 0.174 for the second trimester. MoM SP1 also did not correlate significantly with anti- β_2 -GP I in both trimesters. The correlation between anti- β_2 -GP I IgG and oLAb IgG was not significant ($r = -0.06$). In the first trimester 40 % serum samples were positive for anti- β_2 -GP I IgG and negative for oLAb IgG or *vice versa*, while 60 % samples in the second trimester were positive only for one determined autoantibody. We can conclude that the levels of anti- β_2 -GP I IgG decrease during the second trimester probably as the result of the effects of some immunosuppressive agents associated with pregnancy. The finding of negative correlation between AFP and anti- β_2 -GP I suggests that anti- β_2 -GP I has an influence on fetus development.

Key words

Anti- β_2 -glycoprotein I • Antiphospholipid antibodies • α_1 -fetoprotein • Human chorionic gonadotrophin • Trophoblast-specific - β_1 -glycoprotein • Pregnancy

Introduction

β_2 -glycoprotein I (β_2 -GP I) is a plasma protein of molecular weight about 50 kDa with a carbohydrate content of 17 %. It is present in normal human plasma at approximately 200 mg/l (Schultze *et al.* 1961). The molecule of β_2 -GP I is composed of 326 amino acids with a high proportion of proline, cysteine and tryptophan residue (Lozier *et al.* 1984). The secondary structure of mature β_2 -GP I is formed by five repeating domains of about 60 amino acids, containing highly conserved half-cysteine residues. They are called short consensus repeats (SCRs) (Kato and Enjyoji 1991, Matsuura *et al.* 1991).

The physiological role of β_2 -GP I *in vivo* is not quite clear. *In vitro* β_2 -GP I inhibits the contact activation of the intrinsic coagulation pathway, platelet prothrombinase activity and ADP-induced platelet aggregation (Schousboe 1985, Nimpf *et al.* 1986, 1987). It has also an inhibitory effect on activated protein C activity (Ieko *et al.* 1999).

β_2 -GP I binds to negatively charged substances such as heparin or DNA and to negatively charged phospholipids. About 35 % of β_2 -GP I is present in the lipoproteins - chylomicrons, very low-density lipoproteins and high-density lipoproteins as apolipoprotein H (Kroll *et al.* 1976, Polz and Kostner 1979).

It is known that binding of β_2 -GP I to anionic phospholipids is thought to be the major antigen required in the reaction of antiphospholipid antibodies (APL) to phospholipids. There are several epitopes present on β_2 -GP I. APL may recognize an epitope on a conformationally changed β_2 -GP I arising in its interaction with negatively charged phospholipids. Another possible epitope may be a part of a complex formed by β_2 -GP I and a phospholipid (McNeil *et al.* 1990, Chamley 1997).

Maatsura *et al.* (1992) distinguished non-specific antibodies generated in response to infection that only concern phospholipids and antibodies binding β_2 -GP I that are thus of an autoimmune nature and thus associated with the prothrombic predisposition. Similarly, Forastiero *et al.* (1996) observed that IgG anticardiolipin antibodies (ACA) from patients with the antiphospholipid syndrome did not bind ACA in the absence of β_2 -GP I but recognized β_2 -GP I on irradiated plates in the absence of phospholipids. On the contrary, IgG ACA purified from syphilis patients were bound to cardiolipin alone. In this way it could be possible to distinguish ACA from autoimmune or infectious diseases.

The aim of this study was to investigate the changes of anti- β_2 -GP I IgG during the first and second trimester of pregnancy and the relationship between the levels of anti- β_2 -GP I and fetoplacental antigens – α_1 -fetoprotein (AFP), human chorionic gonadotropin (HCG) and the trophoblast-specific- β_1 -glycoprotein (SP1). We have also investigated the correlation between anti- β_2 -GP I IgG and antibodies against oxidized low-density lipoprotein IgG (oLAB) in the serum of pregnant women. This study is a continuation of our previous report dealing with the oLAB in the first and second trimester of pregnancy and their relationship to fetoplacental antigens (Fialová *et al.* 2002).

Methods

Patients

We studied 204 pregnant women attending antenatal clinic. Routine blood sampling was performed in the first trimester (9-12th week of pregnancy) and in the second trimester (16-18th week of pregnancy). Their mean age was 27 ± 4.1 years.

Sera were collected from nonheparinized blood samples. Venous blood was withdrawn in the morning after an overnight fast. Anticardiolipin antibodies IgG and maternal serum (MS) levels of AFP, HCG and SP1 were determined in fresh serum samples from pregnant women. From this group we selected 52 serum samples positive for ACA IgG (group 1) and 16 samples negative for ACA IgG (group 2). We determined levels of anti- β_2 -GP I IgG and oLAB IgG in the samples of this group.

Determination of anti- β_2 -GP I IgG

Antibodies against β_2 -GP I IgG were assayed by anti- β_2 -GP I IgG commercial ELISA kit (ORGenTec, Germany) using γ -irradiated polystyrene microtitre plates which are coated by highly purified human β_2 -GP I. Diluted patient's samples are pipetted into the wells and incubated for 30 min at room temperature. After washing an anti-human-IgG horseradish peroxidase conjugate solution is added into each well and incubated for 15 min at room temperature. The non-specifically bound enzyme conjugate is washed away and then tetramethylbenzidine is added as substrate. After 15 min incubation at room temperature the color reaction is stopped by adding a hydrochloric acid solution. Samples were considered positive if the levels of antibodies exceeded 8 U/ml.

Determination of other analytes

Anticardiolipin antibodies were examined by the modified ELISA method utilizing microplates coated with highly purified cardiolipin (Sigma Chemicals) (Harris 1990).

For determination of oLAB we used kit oLAB-ELISA (Biomedica, Wien). The microtiter strips were coated by Cu^{2+} oxidized LDL as antigen. The levels of IgG oLAB higher than 800 mU/ml were considered to be positive.

Maternal serum levels of AFP and HCG were assayed by ELISA kits (Sevapharma a.s., CR). MS SP1 were analyzed using single radial immunodiffusion with Q-antiserum (SwAHu SP1, Sevapharma, a.s. CR). The results were expressed as multiples of median values (MoM) for each week of pregnancy.

Statistical methods

The relationship between anti- β_2 -GP I IgG serum levels and oLAB IgG and MS AFP, MS HCG and MS SP1 was explored by calculating the Spearman correlation coefficient. The changes of anti- β_2 -GP I IgG levels in the first and second trimester were calculated using the paired non-parametric Wilcoxon test.

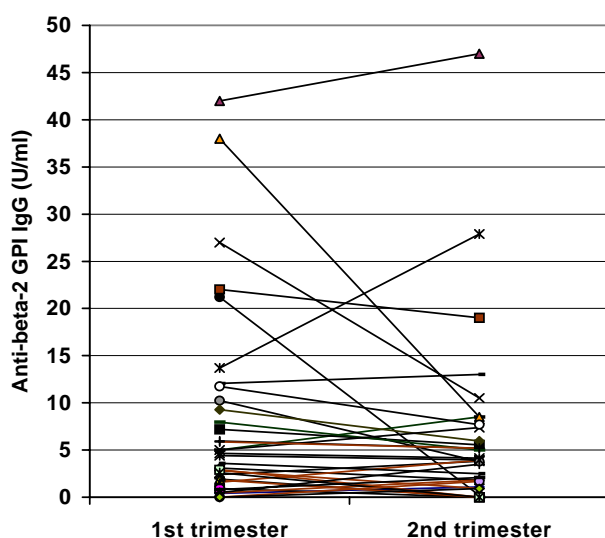


Fig. 1. Individual maternal serum levels of anti- β_2 -GP I IgG in pregnant women. Comparison between first and second trimester. The lines connect the values of anti- β_2 -GP I in the first and second trimester in 34 different women.

Results

Anti- β_2 -GP I IgG levels of patients in the group 1 and 2 significantly decreased in the second trimester. The anti- β_2 -GP I level was 8.3 ± 10.4 U/ml in the first trimester and 6.2 ± 9.3 U/ml in the second trimester ($p=0.05$) (Fig. 1).

Table 1 shows the Spearman's correlation coefficients between anti- β_2 -GP I IgG and particular fetoplacental antigens in the first and second trimester. MoM AFP correlated negatively but not significantly in the first trimester with anti- β_2 -GP I ($r = -0.261$, $p=0.12$). In the second trimester the correlation was significantly negative ($r = -0.278$, $p=0.04$). The Spearman correlation coefficients for MoM HCG were $r = 0.158$ for the first trimester and $r = 0.174$ for the second trimester. MoM SP1 also did not correlate significantly in both trimesters.

Maternal serum levels of AFP, MS HCG and MS SP1 in the anti- β_2 -GP I IgG positive women from the group 1 and 2 are shown in Table 2. In these women, we found both high and low levels of fetoplacental antigens beside normal values. Because of the low number of anti- β_2 -GP I positive women, the results were not statistically evaluated. The value of AFP (MoM) ≥ 2.5 , HCG (MoM) ≥ 2.5 , SP1 (MoM) ≥ 1.5 were considered to be high; the value of AFP (MoM), HCG (MoM) and SP1 (MoM) ≤ 0.5 were considered as low.

The correlation between anti- β_2 -GP I IgG and oLAB IgG is not statistically significant ($r = -0.06$). In the first trimester 40 % serum samples were positive for anti- β_2 -GP I IgG and negative for oLAB IgG or *vice versa*, in the second trimester the 60 % samples were positive only for one determined autoantibody.

Discussion

β_2 -GP I is mainly synthesized in the liver (Koike 2000). During pregnancy β_2 -GP I is also produced by the placenta. mRNA for β_2 -GP I has been identified in extracts of villous placental tissue and the choriocarcinoma cell lines (Chamley *et al.* 1997). Protein production was confirmed by immunoblotting. The presence of β_2 -GP I was demonstrated by immunohistochemistry and by elution experiments in the syncytiotrophoblast and extravillous cytotrophoblast of both normal term placentae and placentae from pregnancies affected by antiphospholipid antibodies (APL) (Chamley *et al.* 1993, 1997). The placenta is one of the important target organs in some autoimmune conditions and β_2 -GP I could form the optimal antigenic

epitope recognized by circulating APL on the cell surface. These observations indicate that β_2 -GP I may possess a physiological function in the placenta (Chamley *et al.* 1997). The syncytiotrophoblast β_2 -GP I may play an

important role in uteroplacental hemostasis during pregnancy. APL may induce their pathological effect by interfering with the function of β_2 -GP I of placental origin

Table 1. Spearman's correlation coefficients between anti- β_2 -GP I IgG and fetoplacental antigens

Correlation of β_2 -GP I and antigens	First trimester		Second trimester	
	r	p	r	p
AFP (MoM)	- 0.261	0.120	- 0.278	0.040*
HCG (MoM)	0.158	0.367	0.174	0.331
SP1 (MoM)	0.125	0.480	- 0.085	0.630

*Statistically significant

Table 2. Maternal serum levels of AFP, HCG and SP1 in anti- β_2 -GP I positive women

Patient No.	First trimester					Second trimester				
	anti- β_2 -GP I (U/ml)	oLAb (mU/ml)	AFP* (MoM)	SP1* (MoM)	HCG* (MoM)	anti- β_2 -GP I (U/ml)	oLAb (mU/ml)	AFP* (MoM)	SP1* (MoM)	HCG* (MoM)
1	38.0	115	1.53	0.72	1.08	8.5	90	1.80	1.30	1.40
2	27.0	510	1.80	2.40	0.75	10.5	619	1.37	1.75	0.49
3	10.2	1330	0.47	0.88	0.93	3.8	1389	0.80	1.25	0.50
4	11.8	1167	1.08	1.22	0.81	7.7	1267	0.66	0.92	0.53
5	12.4	1029	0.89	1.01	1.30	12.9	1060	0.74	0.95	1.08
6	9.28	1458	0.88	1.08	0.67	5.9	1405	0.80	1.15	0.73
7	22.0	349	1.01	1.39	1.50	19.0	305	0.99	1.28	1.60
8	42.0	1190	0.86	0.64	0.72	47.0	1089	1.06	0.42	1.15
9	13.7	-	1.28	0.53	1.29	27.9	-	0.80	0.65	0.55
10	21.2	-	0.83	0.75	1.12	17.0	-	1.23	0.86	0.74

Because of the low number of anti- β_2 -GP I positive women the results were not statistically evaluated. * The values of AFP (MoM) ≥ 2.5 , HCG (MoM) ≥ 2.5 , SP1(MoM) ≥ 1.5 were considered as high; the values of AFP (MoM), HCG (MoM) and SP1 (MoM) ≤ 0.5 were considered as low.

Recently George *et al.* (1999) proposed the hypothesis that APL known to induce a thrombotic tendency also possesses proatherogenic properties because these antibodies have been shown to display endothelial and platelet activating properties *in vitro*. They have found β_2 -GP I to be distributed in all layers of the atherosclerotic plaque, intracellularly and extracellularly. β_2 -GP I colocalized with CD 4 lymphocytes supporting its role as a target for mediated inflammatory effector cells. These findings have led to the assumption that the immune response against β_2 -GP I

is involved in the development of inflammation within atherosclerotic plaques.

APL and β_2 -GP I may contribute to pregnancy complications by enhancing atherogenesis in spiral arteries. Acute atherosclerosis can cause partial or complete blockade of spiral arteries which can lead to thrombus formation and placental infarction (Redman 1991). Moreover, it has recently been reported that β_2 -GP I was recognized by APL when bound to oxidized phospholipids. This indicates that some APL are in fact directed to epitopes of oxidized phospholipids, or

neoepitopes generated by adduct formation between breakdown products of oxidized phospholipids and associated proteins (including β_2 -GP I) (Hörkkö *et al.* 1997). It is interesting that oxidized low-density lipoproteins (oxLDL) are an important factor in the initiation and progression of atherosclerosis (Steinerová *et al.* 2001). Since plasma β_2 -GP I is directly bound to oxidized plasma lipoproteins, the antibodies to β_2 -GP I may react with oxidized LDL (Koike 2000).

Another function of β_2 -GP I in reproduction was proposed by Chamley (1997), who speculated that this protein may act as an adhesion molecule interacting with heparin sulphate of the extracellular matrix and phospholipids on the surface of trophoblast cells. This hypothesis is supported by some findings in mice demonstrating that APL not only bind to the trophectoderm of preimplantation embryos, but can also prevent implantation in an *in vitro* model. Embryos obtained from ACA-immunized females were severely impaired. It is thus possible that APL can disrupt the interaction of trophoblast cells with the decidualized endometrium (Stoeger *et al.* 1993). β_2 -GP I may bind to cell surfaces *via* interaction with negatively charged phospholipids of the cell membrane, or with cell surface heparin-like molecules or *via* a cell surface receptor (Chamley *et al.* 1993, 1997). Direct binding of APL to placental tissue may disrupt uteroplacental blood flow and/or transport through the villi (Chamley *et al.* 1993).

Recent studies have suggested that APL decreased hormone production by placenta and trophoblast intracellular fusion and invasion. These facts indicate that some of the pregnancy complications observed in the antiphospholipid syndrome may be caused by trophoblast dysfunction induced by antiphospholipid antibodies (Caruso *et al.* 1999). Both polyclonal and monoclonal APL significantly reduced HCG release *in vitro*. This finding suggests that APL recognition of both anionic phospholipids and adhered β_2 -GP I on trophoblast cell structures might represent a potential pathogenetic mechanism for defective placentation in women with the antiphospholipid syndrome (DiSomone *et al.* 2000).

We did not observe a decrease of placental proteins in the APL positive women *in vivo*. The correlation between anti- β_2 -GP I levels and HCG and SP1 was not significant. We found low, normal and high

levels of HCG and SP1 in anti- β_2 -GP I positive women. There is another situation for protein produced by fetus – α_1 -fetoprotein. The finding of statistically significant negative correlation may suggest a relationship between anti- β_2 -GP I and AFP. This observation may explain the fact that the production of some agents with immunosuppressive effects increase during pregnancy. High levels of AFP can act immunosuppressively for the biosynthesis of autoantibodies. It may also be a reason of anti- β_2 -GP I decrease in the second trimester of pregnancy.

High concentration of APL has been eluted from the placenta of APL positive women with late pregnancy complications (Katano *et al.* 1995). The vasculosyncytial membrane is porous enough for IgG antibodies and APL may penetrate into the fetal circulation after 15th week of pregnancy. Adverse effects of anti- β_2 -GP I to the fetus development cannot be excluded.

Similarly as Tinahones *et al.* (1998) in the group of patients with antiphospholipid syndrome, we were unable to demonstrate a correlation between oLAb and anti- β_2 -GP I in the serum of pregnant women. We found autoantibodies reacting to only one antigen – oxLDL or β_2 -GP I in some serum samples and in other samples antibodies binding both antigens. This finding suggests that there are different populations of antibodies – antibodies that react specifically with β_2 -GP I, antibodies reacting only with oxidized LDL and cross-reacting antibodies. It cannot be excluded that various kinds of autoantibodies may act in different ways.

We can conclude that the levels of anti- β_2 -GP I IgG decrease during the second trimester probably as the result of the effects of some immunosuppressive agents associated with pregnancy. The negative correlation between AFP and anti- β_2 -GP I suggests that anti- β_2 -GP I probably influences fetus development. The low maternal serum levels are associated with an increased risk of Down's syndrome of the fetus and this fact should therefore be taken into account in the interpretation of the results of prenatal biochemical screening of congenital chromosomal abnormalities.

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