Pathogenic role of anti-beta 2 glycoprotein I antibodies in antiphospholipid-associated fetal loss: characterization of beta 2 glycoprotein I binding to trophoblast cells and functional effects of anti-beta2 glycoprotein I antibodies in vitro

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PATHOGENIC ROLE OF ANTI-BETA 2 GLYCOPROTEIN I ANTIBODIES IN ANTIPHOSPHOLIPID-ASSOCIATED FETAL LOSS: CHARACTERIZATION OF BETA 2 GLYCOPROTEIN I BINDING TO TROPHOBLAST CELLS AND FUNCTIONAL EFFECTS OF ANTI-BETA 2 GLYCOPROTEIN I ANTIBODIES IN VITRO

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Short title: Beta 2 glycoprotein I and trophoblast cells
Key words: Beta2 glycoprotein I, anti-phospholipid antibodies, trophoblast, fetal loss

Some of the data contained in this study were presented at the 3rd International Conference on sex hormones, pregnancy and the rheumatic diseases, New Orleans, October 21-24, 2002.

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ABSTRACT

Objectives: Antiphospholipid antibodies reacting with beta 2 glycoprotein I (β2GPI) have been associated with recurrent fetal loss and pregnancy complications. The aim of the study was to investigate whether specific mutations in the phospholipid (PL)-binding site of β2GPI might affect its binding to trophoblast and in turn the anti-β2GPI antibody-induced functional effects.

Methods: β2GPI adhesion to trophoblast was evaluated as human monoclonal IgM or polyclonal IgG anti-β2GPI antibody binding to trophoblast monolayers cultured: i) in complete medium, ii) in serum-free medium, and after serum starvation iii) in the presence of purified human β2GPI or iv) in the presence of β2GPI with single or multiple mutations in the amino acid loop Cys281 - Lys-Asn-Lys-Glu-Lys-Lys-Cys288. The effect of anti-β2GPI binding to trophoblast was evaluated as chorionic gonadotropin (hCG) mRNA expression and protein release by RT-PCR and radioimmunoassay respectively.

Results: β2GPI adhesion to trophoblast and its consequent recognition by the specific antibodies were inversely proportional to the mutation number in the PL-binding site. Anti-β2GPI antibodies reduced gonadotropin release hormone-dependent hCG mRNA expression and protein synthesis in the presence of β2GPI, while the addition of the mutants or the absence of β2GPI had no effect.

Conclusions: β2GPI binds to trophoblast in vitro through its fifth domain as reported for endothelial cells, and can be recognized by anti-β2GPI antibodies; the antibody binding down-regulates trophoblast hCG synthesis and secretion. Such a mechanism might contribute to the defective placentation in women with the anti-phospholipid syndrome-associated fetal loss.

Key words: trophoblast, β2-glycoprotein I, antiphospholipid antibodies, human chorionic gonadotropin, fetal loss.
INTRODUCTION

Human β2-glycoprotein I (β2GPI) is a phospholipid-binding plasma protein that represents one of the main antigenic targets for antiphospholipid antibodies (aPL), the formal diagnostic tools for the anti-phospholipid syndrome (APS) [1-5]. Beta 2GPI-dependent aPL not only display a diagnostic value but also play a pathogenic role in APS [6].

Beta2GPI is composed of five highly conserved subunits called sushi domains or complement control protein repeats [7]. The molecule binds to negatively charged structures through a major PL-binding site located in the fifth domain, and identified as a highly positively charged amino acid sequence: Cys281-Lys-Asn-Lys-Glu-Lys-Lys-Cys288 [8].

Anti-β2GPI antibodies recognize different epitopes located in all five domains, however several groups published that the majority of the antibodies are directed against domain I [reviewed in 7]. Recent findings suggest that the antibodies display low avidity and react with the native β2GPI when the molecule is available at increased antigenic density [9-11].

Beta2GPI binds not only to negatively charged substances such as PLs, heparin, DNA and lipoproteins, but also to surface membranes of cell types directly involved in the APS pathogenic mechanisms, such as activated platelets and endothelial cells [7,12,13]. It has been recently reported that dimeric β2GPI binds to anionic PLs exposed on activated platelets and interacts with apolipoprotein E receptor 2, a member of the low density lipoprotein receptor family expressed on both platelets and trophoblast [14,15]. The PL-binding site in the fifth domain of the molecule was also shown to be involved in the binding of the molecule to endothelial cell membranes [16].

More recently, we found that β2GPI can adhere to human trophoblast cells in vitro [17]. Our results are consistent with the hypothesis that the exposure of anionic PLs on the external cell surface during intertrophoblastic fusion might offer a useful substrate for the cationic PL-binding site [18,19]. This finding is in line with the previous immunohistological demonstration of the in vivo binding of β2GPI to trophoblast structures [20,21]. Once bound, the molecule offers suitable epitopes for both human polyclonal and monoclonal anti-β2GPI antibodies obtained from APS patients. The antibody binding was found to affect trophoblast differentiation in vitro by inhibiting gonadotrophin release and matrigel invasiveness, which might represent mechanisms potentially involved in the defective placentation reported in APS [17].

By using recombinant β2GPI mutants we investigated whether β2GPI could bind to trophoblast cells in a manner comparable to that shown for CL-coated plates and endothelial monolayers and whether such a binding is strictly required for the antibody-mediated biological effects on trophoblast.
MATERIALS AND METHODS

Human β2GPI Purification. Human β2GPI was purified from human serum and characterized as previously described [22].

Human β2GPI Mutants Site-directed mutagenesis was performed to assess the role of individual amino acids in the Cys\(^{281}\)-Cys\(^{288}\) loop for the PL-binding and the cofactor activity as detailed [8,23]. Four mutants were obtained: 1K, with a single amino acid change from Lys\(^{286}\) to Glu\(^{286}\); 2K and 2Ka, with a double amino acid change (from Lys\(^{286}, 287\) to Glu\(^{286}, 287\) and from Lys\(^{284}, 287\) to Glu\(^{284}, 287\) respectively); and 3K with a triple amino acid change (from Lys\(^{284}, 286, 287\) to Glu\(^{284}, 286\) and \(^{287}\)). In comparison to human purified β2GPI, mutant 1K displayed: 1) reduced PL-binding activity, 2) lower cofactor activity for β2GPI-dependent antibodies in a CL-ELISA, and 3) decreased inhibition of the binding of iodinated human purified β2GPI to CL-coated plates. Double or triple mutants 2K, 2Ka and 3K lost the PL-binding activity and did not display any cofactor activity for anti-β2GPI antibodies in CL ELISA or the ability to inhibit iodinated native β2GPI binding to CL. While mutations in the Lys\(^{282-287}\) loop altered the properties of the PL-binding site, they did not affect the recognition of all the mutants by purified polyclonal anti-β2GPI antibodies [23]. Comparable experiments carried out with the mAbs gave similar results (data not shown).

Human Monoclonal anti-β2GPI Antibodies Two human monoclonal antibodies (mAbs) of the IgM isotype obtained from hybridised Epstein-Barr virus (EBV)-induced B cell lines from APS patients were used. TM1G2 has been shown to recognize β2GPI both complexed with anionic PL (in CL-coated plates) and alone in γ-irradiated β2GPI-coated plates. TM1B9 did not display any anti-β2GPI reactivity and was used as a negative control. The characterization of the mAbs had been previously reported in detail [24].

Human Polyclonal anti-β2GPI Antibodies. Whole IgG fractions from two patients suffering from primary APS diagnosed according to the Sapporo criteria [25] and from two normal human sera (NHS) were purified on Protein-G-Sepharose [22]. The final protein IgG concentration was evaluated by nephelometry and their specific reactivity with β2GPI-coated plates was confirmed as previously described [22].

Cell Cultures Placentas were obtained from healthy women immediately after uncomplicated vaginal delivery at 36 weeks of gestation. Cytotrophoblast cells were isolated as detailed elsewhere [26]. The enriched (95%) cytotrophoblast cells (5x10^5 cells/ml) were cultured in DMEM-10% FCS in 96-well plates at 37°C in 5% CO\(_2\)/95% air. The purity and the maturation of the cell preparations were evaluated by using a panel of antisera directed against fibroblasts, macrophages, cytocheratin and hCG as previously described [17, 26]. 95% of the cell preparations tested positive for anti-cytocheratin antibodies. Cytotrophoblasts at different times of culture were further assayed for the cytoplasmic presence of hCG as a marker for syncytial trophoblast. Cell cultures were performed for 72 hours in standard medium, washed three times with HBSS (Hank's Balanced Salt Solution, Flow Laboratories, Irvine, U.K.) and cultured in serum-free (HyQ-CCMTM 1, HyClone, Laboratories, Logan, UT) to remove adherent β2GPI [17].
Binding Assays

On day 3 (72 hours) of culture in 96 well plates, the medium was removed, the cells washed and cultured in serum-free medium. A cell-ELISA was performed to determine whether the polyclonal and monoclonal anti-\(\beta_2\)GPI antibodies bound to trophoblast cells through the adherent \(\beta_2\)GPI and \(\beta_2\)GPI mutants as previously described [17]. Serum-free trophoblast cell cultures were incubated for 1 hour at 37°C with different protein concentrations of: i) human purified \(\beta_2\)GPI (5, 2.5, 1.25 \(\mu\)g/ml); ii) recombinant \(\beta_2\)GPI mutants 1K, 2K, 2Ka and 3K (5, 2.5, 1.25 \(\mu\)g/ml); or iii) serum-free medium. Polyclonal or monoclonal anti-\(\beta_2\)GPI antibodies (50 \(\mu\)g/ml) were added to the wells. After 2-hour incubation, followed by three washes, the plates were incubated with alkaline phosphatase-conjugated goat anti-human IgM or anti-IgG (Sigma Chemicals, St Louis, MO, USA) for 90 minutes. After two further washes, p-nitrophenylphosphate (1 mg/ml) in 10% diethanolamine buffer, pH 9.8, was added to each well and incubated for 30 minutes. O.D. was read at 405 nm by a microplate photometer (Plettreader; Bio-Rad Laboratoires, S.r.l., Milano, Italy) [17].

Hormone Secretion

Human purified \(\beta_2\)GPI or \(\beta_2\)GPI mutants (5 \(\mu\)g/ml) were added to primary trophoblast cells cultured in serum-free medium. After 1-hour incubation, 50 \(\mu\)g/ml of polyclonal or monoclonal anti-\(\beta_2\)GPI antibodies or appropriate controls were added. The culture media were changed daily from the first day of incubation. After 72-hour culture, the cells were then treated for 24 hours with 10\(^{-7}\) M gonadotropin-releasing hormone (GnRH; Lutrelef, Ferring, Milano, Italy). At the end of the incubation, the media were removed and stored at –20°C for human chorionic gonadotropin (hCG) determination. The assay was performed with a commercial radioimmunoassay kit (Radim, Roma, Italy) as described [17]. The intra- and inter-assay coefficients of variation were < 12 and < 8%, respectively.

Semiquantitative RT-PCR Analysis

A comparable experimental protocol has been carried out to evaluate the hCG mRNA expression on trophoblast cell cultures. Confluent cells were collected, centrifuged, washed and total RNA was isolated by lysing cells with Trizol\textsuperscript{TM} Reagent (GIBCO BRL) according to manufacturer’s instructions. RNA integrity was confirmed by agarose gel electrophoresis and ethidium bromide staining as well as by monitoring absorbance at 260/280 nm. The RNA concentration was determined by spectrophotometric analysis and before each RT-PCR experiment. The Perkin-Elmer Gene Amp Gold RNA PCR kit was used for all the RT-PCRs, which were performed in the Gene Amp PCR system 9600 (Perkin-Elmer/Cetus, San Diego, CA). After removal of contaminating chromosomal DNA with DNAse I treatment, 1 \(\mu\)g of RNA was reverse-transcribed (RT) with 25 units of Moloney murine leukemia virus at 42°C for 20 minutes. Three \(\mu\)l of cDNA products were used in each PCR cycle. Primer sequence and location as well as expected product size are listed in Table 1.
Table 1. Sequence and expected fragment size for synthetic oligos used for PCR and Southern blotting.

<table>
<thead>
<tr>
<th>Oligo Sequence</th>
<th>Primer Sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-hCG5’</td>
<td>ATG-CCA-CCC-TGG-CTG-TGG-AGA-A</td>
<td>367</td>
</tr>
<tr>
<td>β-hCG3’</td>
<td>GGG-AGT-CGG-GAT-GGA-CTT-GGA-A</td>
<td></td>
</tr>
<tr>
<td>β-hCG-Southern</td>
<td>CAA-CTA-CCG-CGA-TGT-GCG-C</td>
<td></td>
</tr>
<tr>
<td>Aldolase5’</td>
<td>CGC-AGA-AGG-GGT-CCT-GGT-GA</td>
<td>176</td>
</tr>
<tr>
<td>Aldolase3’</td>
<td>CAG-CTC-CTT-CTT-CTG-CTC-CGG-GGT</td>
<td></td>
</tr>
<tr>
<td>Aldolase-Southern</td>
<td>GAA-CTT-GCT-ACT-ACC-AGC-A</td>
<td></td>
</tr>
</tbody>
</table>

The hCG cDNA was co-amplified with aldolase-A as internal control with 1 unit of AmpliTaq Gold DNA polymerase in 1.75 mM MgCl2, and 32 cycles of 20 sec at 94°C, 60 sec at 62°C followed by 7 min at 72°C. The PCR products were loaded onto 2% agarose gels and stained with ethidium bromide. The images of the gels were acquired with a Cohu charged coupled device camera, and quantification was performed with Phoretix ID (Phoretix International Ltd., Newcastle upon Tyne, UK). The relative concentration of each hCG mRNA was determined by densitometric scanning and normalization to the aldolase-A signal for each sample. The number of cycles and the reaction conditions were chosen so that none of the target cDNAs reached a plateau and that the two pairs of primers did not compete with each other. The PCR product identity was verified by Southern blot using synthetic oligos located internal to the two PCR primers (Table 1).

Statistical Analysis

Statistical analysis were performed using Student’s t test and two-way analysis of variance for multiple comparisons; P values <0.05 were considered significant.
RESULTS

Binding of human purified β2GPI and β2GPI mutants to trophoblast cells.

In order to demonstrate the role of β2GPI or mutants in the anti-β2GPI antibody trophoblast binding, cell cultures have been extensively washed and cultured in serum-free medium to remove adherent β2GPI. In such conditions, TM1G2 showed background binding values comparable to those found with the irrelevant control TM1B9. Addition of exogenous human β2GPI restored TM1G2 (50 µg/ml) but did not affect TM1B9 (50 µg/ml) binding (data not shown). When trophoblast cells were incubated with 1K, 2K and 2Ka mutants (5 µg/ml), TM1G2 binding values were 59 ± 3, 41 ± 5, and 35 ± 3% respectively (mean ± SD; n=3 experiments) of those obtained in the presence of human purified β2GPI. Lower TM1G2 binding (26 ± 2%, mean ± SD; n=3 experiments) was observed when trophoblast cells were incubated in the presence of 3K mutant (Figure 1). TM1G2 mAb binding to trophoblast was dependent on the final amount of human purified β2GPI or recombinant mutants added to the cultures (Figure 1).

Comparable results were found by using whole polyclonal IgG fractions (50 µg/ml) with anti-β2GPI activity but not with NHS IgG (data not shown).

All of the human antibodies were used at a final protein concentration of 50 µg/ml, which has been previously shown to display optimal binding [17].

Modulation of hCG protein secretion by anti-β2GPI antibodies.

In accordance with previous findings, addition of GnRH (10⁻⁷M) to trophoblast cells significantly increased hCG secretion [17]. The incubation of trophoblast cells with NHS IgG in the presence of human β2GPI (5 µg/ml) did not modify both basal and GnRH-induced hCG production (data not shown). In contrast, polyclonal anti-β2GPI IgG (50 µg/ml) significantly reduced GnRH-induced hCG secretion when the cells were cultured in the presence of human purified β2GPI (5 µg/ml); no changes in basal and GnRH-induced hormonal placental secretion were observed in the presence of 3K mutant (5 µg/ml) or in serum-free conditions (Fig 2A). Experiments carried out with the human anti-β2GPI IgM mAb (TM1G2, 50 µg/ml) gave comparable results (31±4, 12±4, 28±3 hCG mIU/ml m±3SD of triplicate experiments for cultures performed in the presence of GnRH plus serum-free medium or native β2GPI or 3K mutant respectively; 11±3, 10±2, 10±4 hCG mIU/ml m±3SD of triplicate experiments for comparable cultures carried out without GnRH). Cultures performed in the presence of the irrelevant control (TM1B9; 50 µg/ml) gave values ranging from 29 to 32 hCG mIU/ml in the presence of GnRH and values < 13.5 hCG mIU/ml in the absence of GnRH.

Figure 2B reports the results on the hCG secretion by trophoblast monolayers incubated in the presence of mAbs and the mutants. TM1G2 mAb recognized 1K mutant and induced an inhibition of hCG secretion lower that that displayed in the presence of native β2GPI but still statistically significant in comparison to the control. Mutant 2Ka as well as 3K were not recognized and no inhibition of hCG secretion was found.

Incubation of trophoblast cells with human β2GPI or 1K, 2Ka and 3K mutants (5 µg/ml) alone did not modify basal (<13.5 hCG mIU/ml) or GnRh-induced hCG production (28±3, 29±2, 31±5, 30±4 hCG mIU/ml respectively).

Modulation of hCG mRNA expression by polyclonal anti-β2GPI IgG.

Since it has been demonstrated that both polyclonal and monoclonal anti-β2GPI Abs down-regulate the GnRH-induced hCG secretion after trophoblast binding, we investigated whether such an effect might be reproducible also at the hCG mRNA level.
Addition of GnRH \((10^{-7} M)\) to trophoblast cells significantly increased hCG mRNA expression. As shown in Figure 3, the presence of both anti-\(\beta2\)GPI IgG \((50 \mu g/ml)\) and human purified \(\beta2\)GPI \((5 \mu g/ml)\) significantly reduced GnRH-induced hCG mRNA expression. In parallel experiments performed with 3K mutant, GnRH-induced hCG mRNA was not inhibited by the addition of anti-\(\beta2\)GPI IgG. Incubation with NHS IgG did not modify hCG mRNA expression in all the conditions (data not shown).

Comparable results have been found by using TM1G2 anti-\(\beta2\)GPI mAb and its irrelevant control (TM1B9) (data not shown).
DISCUSSION

Our results show for the first time that β2GPI adhesion to trophoblast cell membranes involves the same PL-binding site used for the binding to negatively charged structures.

It has been suggested that the highly positively charged amino acid sequence, Cys281-Lys-Asn-Lys-Glu-Lys-Lys-Cys288, in the fifth domain of the molecule is the putative PL-binding site responsible for the β2GPI binding to CL-coated plates. Single or multiple amino acid substitutions of Lys with Glu progressively decrease the ability of the molecule to bind to anionic structures [8,23]. Interestingly, the same PL-binding site is involved in the adhesion of β2GPI to human endothelial cell monolayers, since Lys substitution with Glu significantly decrease the presence of β2GPI on endothelial monolayers as shown by the lack of anti-β2GPI Ab binding [16].

We confirmed our previous results by showing that human purified β2GPI binds to trophoblast cells and it is recognized by both monoclonal and polyclonal anti-β2GPI Abs from APS sera [17]. When trophoblast cells were incubated with serial protein concentrations of mutant 1K (single amino acid substitution from Lys286 to Glu286) approximately 50% reduction of anti-β2GPI antibodies bound to the cells in comparison with trophoblasts cultured with comparable protein concentrations of purified β2GPI. Much lower antibody binding was found by using 2K (substitution from Lys286,287 to Glu286,287) and 2Ka (substitution from Lys284,287 to Glu284,287) mutants and the lowest antibody binding was detected with the mutant 3K (substitution from Lys284,286,287 to Glu284,286,287).

We previously reported that, once bound to trophoblast adhered β2GPI, anti-β2GPI antibodies might significantly inhibit GnRH-induced hCG protein release from trophoblast cell cultures [17]. We confirmed and extended the previous data by showing that anti-β2GPI antibody-mediated hCG inhibition might occur at the mRNA level too. Moreover, the present findings indicate that large alteration of the PL-binding site on the fifth domain of the molecule does not allow an efficient β2GPI adhesion, antibody binding and in turn the antibody-mediated cell function modulation. Actually, experiments carried out with trophoblast cells incubated with anti-β2GPI antibodies and mutant 3K display hCG mRNA expression comparable to those found in control or serum-free cultures.

Cytotrophoblast cells from term placentas were used because of ethical reasons. Although they do display lower percentage of mitotic figures and lower in vitro invasiveness than cells taken from the first or second trimester placentas, nevertheless they were previously shown to be a suitable in vitro experimental tools for investigating aPL-mediated effects [17].

The presence of β2GPI on the trophoblast cell membranes might be one of the main targets for β2GPI-dependent aPL in the placental circulation [20]. Such a finding does represent the prerequisite for supporting a pathogenic role for these antibodies as suggested by the clinical association between recurrent fetal loss and β2GPI-dependent aPL and/or anti-human β2GPI antibodies themselves [5, 27-30]. At the same time, it also might explain the aPL placental tropism that has been described in experimental animal models of aPL-associated fetal loss. Actually, when exogenous human aPL are passively infused in pregnant naïve mice they undergo a rapid plasma clearance [31,32]. It has been suggested that the fast plasma clearance could be related to the aPL binding to placental structures since the same Abs can be eluted from them [32]. Moreover, there is also evidence from immunohistochemical studies that β2GPI is expressed at higher quantity on the trophoblast villi of placentas from APS women with fetal loss than in controls and that an Ig deposition with a comparable immunohistochemical pattern is also detectable [21]. These findings do suggest that most of the circulating β2GPI-dependent aPL (or even the anti-β2GPI antibodies themselves) might be bound to placental β2GPI in vivo. The fact that aPL can be “absorbed” by placental structures has been thought to be pivotal for allowing the potential pathogenic effect of the antibodies on the placenta and to explain - at least in part - why maternal IgG aPL do not cause so frequently thrombotic events in the fetuses or neonates [33].
Our results are consistent with the hypothesis that \( \beta 2GPI \) binds to trophoblast cell membranes through the PL-binding site in the fifth domain of the molecule being able to trap or to increase antigen density. The exposure of anionic PLs on the cell membranes during intertrophoblastic fusion might represent the natural substrate for placental \( \beta 2GPI \) adhesion [18,19].

It is possible to speculate that the clustering effect of anti-\( \beta 2GPI \) Abs on trophoblast adhered \( \beta 2GPI \) might be the molecular event that signals the hCG mRNA down-regulation as well as the impaired invasiveness [17], eventually contributing to the defective placentation in APS [34]. It has been reported that an anti-phosphatidylserine mAb is able to inhibit intracellular fusion of a choriocarcinoma cell line and we found comparable results by incubating polyclonal IgG from APS patients with anti-\( \beta 2GPI \) binding activity on cytotrophoblast cells [35,36]. So, it is possible that anti-\( \beta 2GPI \) antibodies might affect placental development also by interfering with the normal syncitiotrophoblast formation, being the reduced hCG secretion in the presence of anti-\( \beta 2GPI \) antibodies the result of trophoblast fusion process inhibition.
ACKNOWLEDGMENTS

This study was in part supported by Ricerca Corrente 2000-02 IRCCS Istituto Auxologico Italiano (to PLM), by research grant from the Catholic University of Rome (D1, year 2002; to AC), and by the NH & MRC Australia (to SAK).
LEGENDS OF THE FIGURES

Figure 1: Human anti-β2GPI mAb binding to trophoblast cell monolayers.

Cell cultures were incubated with TM1G2 mAb (50 µg/ml) in the presence of human purified β2GPI (◇), 1K (▧), 2K (▤), 2Ka (▨) or 3K (■) mutants at serial protein concentrations (from 5 to 1.25 µg/ml). Binding values are expressed as mean O.D. units x 10^{-3} ± S.D.; n=3. Washed trophoblast cell monolayers cultured in serum-free medium in the absence of β2GPI or 1K, 2K, 2Ka and 3K mutants and incubated with TM1G2 gave background values of 201 ± 70 O.D. units (mean ± SD of six experiments).

Comparable experiments carried out with TM1B9 mAb (50 µg/ml) gave background-binding values only (values < 200 x 10^{-3} OD units).

TM1G2 mAb binding to trophoblast was significantly higher in the presence of human purified β2GPI than in the presence of mutants (* indicates p < 0.05).

Figure 2: hCG secretion induced by anti-β2GPI polyclonal IgG (A) and by moAbs (B).

A. Trophoblast cell monolayers were incubated in the presence of polyclonal anti-β2GPI IgG (50 µg/ml) and of a) serum-free medium (◼), b) human purified β2GPI (5 µg/ml) (◇), and c) 3K mutant (5 µg/ml) (■). Two sets of cultures carried out respectively in the presence or in the absence of GnRH (10^{-7} M) were performed. hCG values are expressed as mIU/ml. hCG secretion was significantly reduced in cultures performed in the presence of human β2GPI in comparison with cultures carried out with serum-free or 3K mutant (* indicates p < 0.05).

B. Trophoblast cell monolayers were incubated in the presence of GnRH (10^{-7} M) plus TM1G2 moAb (50 µg/ml) and native β2GPI (■) or 1K (▧), 2Ka (▤) or 3K (▦) mutants (5 µg/ml). Control cultures were performed in the presence of GnRH (10^{-7} M) plus TM1B9 moAb (50 µg/ml) and native β2GPI (◇) or 1K (▧), 2Ka (▤) or 3K (▦) mutants (5 µg/ml). hCG secretion by cultures performed in the presence of native β2GPI or 1K mutant plus TM1G2 was significantly reduced in comparison with control cultures carried out with TM1B9 mAb (* indicates p < 0.05). No difference in hCG secretion was found in the cultures performed with the other mutants.

Additional controls were trophoblasts in medium alone (12.3 ± 1.5 hCG mIU/ml; mean ± SD of six experiments) and in the presence of GnRH alone (28.3 ± 1.5 hCG mIU/ml; mean ± SD of six experiments). Cells incubated with mutants alone or in the presence of the moAbs but without GnRH stimulation gave background values (< 13.5 hCG mIU/ml).

Figure 3: hCG mRNA expression induced by anti-β2GPI polyclonal IgG.


B) hCG/aldolase ratio in trophoblast cultures carried out in serum-free, with human β2GPI or 3K mutant and in the presence of anti-β2GPI polyclonal IgG (50 µg/ml). Two sets of cultures have been performed: with (■) and without (◇) GnRH. The results are the mean of five different experiments. Significantly higher hCG/aldolase ratios were found in cultures of trophoblasts in serum-free medium or in the presence of 3K mutant in comparison with cultures carried out in the presence of human β2GPI (* indicates p < 0.05).
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Figure 1
Figure 2
Figure 3

[Image of a gel electrophoresis and a bar graph showing the expression of hCG and Aldolase in different conditions: serum-free, Beta2GPI (5μg/ml), and 3K (5μg/ml). The graph indicates significant differences marked with asterisks.]

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Ann Rheum Dis published online July 15, 2004

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