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Oral administration of Domain-I of beta-2glycoprotein-I induces immunological tolerance in experimental murine antiphospholipid syndrome

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- This study is dedicated to the late Prof. Silvia Pierangeli.

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ABSTRACT

It is well established that the humoral immunity in antiphospholipid syndrome (APS) is presented by circulating pathogenic *anti*-β2GPI autoantibodies targeting mainly domain I of the β2GPI protein, playing a major role in the disease pathogenesis. Previously, we have demonstrated that treatment of experimental APS mice with tolerogenic dendritic cells loaded with domain-I was more efficient in tolerance induction than with the whole molecule or domain-V. In the current study we had orally administered a domain-I derivative of the β2GPI molecule, as a new therapeutic approach to induce oral tolerance in this mouse model of APS. BALB/c mice immunized with β2GPI, were fed with either domain-I, domain-V derivative or the complete β2GPI protein. β2GPI immunized mice developed experimental APS which were fed with domain-I significantly had decreased fetal loss ($p < 0.004$), a lower size of thrombi ($p < 0.001$) and lower circulating *anti*-β2GPI Abs in comparison to mice fed with domain-V or PBS ($p < 0.002$). Likewise, Domain-I fed mice had a lowered inflammatory response, exhibited by decreased expression of inflammatory cytokines (IFNγ, IL-6, IL-17) and elevated production of IL-10 anti-inflammatory cytokine by splenocytes. Moreover, the anti-inflammatory response in the domain-I fed APS mice was associated with increased circulating miRNA variations (155, 146, 182, 98) by RT-PCR, which are associated with immunomodulation of the immune network.

We propose that oral tolerance with domain-I can be a novel therapy for patients with APS.

1. Introduction

The antiphospholipid syndrome (APS) is a coagulopathy presented either by arterial or venous thrombosis and by recurrent fetal loss. In addition systemic involvement of the central nervous system (CNS), heart, skin and other organs have been reported [1–4]. The main APS related circulating antibodies (Abs), target β2-glycoprotein I (β2GPI), these antibodies may be pathogenic or non-pathogenic [5,6]. Exchanging heavy and light chains between pathogenic and non-pathogenic *anti*-β2GPI single-chain Fvs, revealed that the pathogenicity of *anti*-β2GPI Abs lies in the CDR of the heavy chain of the immunoglobulins [7].

Circulating *anti*-β2GPI Abs are heterogeneous populations of Abs targeting different epitopes on domains I^{st} to V^{th} of the β2GPI molecule [8–11]. Groot PG et all [12,13] proved that circular β2GPI is opened during β2GPI-target binding exposing domain-I cryptic immunogenic epitope, to the immune system leading to the generation of anti-domain-I Abs. During the last decade, mounting evidence supports the importance of antibodies targeting the first domain of β2GPI molecule as the main epitope associated with pathogenic features of the disease [13–30]. Treatment of experimental APS with tolerogenic dendritic cells (tDCs) pulsed with domain-I and not domain-V, resulted in tolerance induction, manifested by decreased fetal loss and lower titers of circulating *anti*-β2GPI Abs. Likewise, reduced secretion of proinflammatory cytokines (e.g. IL-1β, IL-12 and IL-23), enhanced IL-10 expression, and expansion of $CD4^+CD25^+FOXP3^+$ T-regulatory cells

Abbreviations: APS, antiphospholipid syndrome; β2GPI, beta 2 glycoprotein-I; DI, domain-I; DV, domain-V; miRNA, microRNA; Treg, T regulatory cell

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(Treg) were recorded [21]. Given the fact that the β2GPI protein can facilitate oral tolerance in experimental APS [31] and that mitigation of the clinical aspects of experimental APS was shown by using domain-I loaded dendritic cells [21], we have attempted to induce oral tolerance with β2GPI-domain-I (D1- β2GPI).

2. Materials and methods

2.1. Mice

Female BALB/c mice at 10–12 weeks and male at 14–16 weeks of age, were purchased from Harlan Israel. The mice were kept in ventilated cages. All experiments were approved and executed according to the protocols of the Ethical Committee of the Israeli Ministry of Health.676/11.

2.2. Induction of experimental APS in mice and treatment

Experimental APS was induced in BALB/c female mice by immunization with human β2GPI 10 μg/mouse or DI- β2GPI 10 μg/mouse (kindly provided by INOVA Diagnostics, USA), in PBS emulsified with Complete Freunds Adjuvant (CFA) injected intradermal in the hind footpads. Oral tolerance was induced when the titer of anti- β2GPI reached plateau at week 6 post immunization by feeding APS mice with affinity purified human β2GPI, DI- β2GPI and synthetic DV- β2GPI derivative GDKVSFFCKNKEKKCKKKK-Ahx-KSSLAFWKTDASDVKP (GL-Biochem Shanghai, China) [21]. N = 20 per each group. The compounds were given orally, weekly, 100μg/mouse.

For fetal loss analyses, the mice were mated and the vaginal plugs were confirmed, as previously described by us [8,9,21,31]. The number of resorptions (equivalent to fetal loss) was counted on day 16 of pregnancy.

In order to assess clotting formation, IgG was affinity purified from mouse sera form APS mice ± oral administration of PBS, β2GPI, DI-β2GPI, DV- β2GPI, on protein-G column. The femoral vein of naïve anesthetized mice were dissected to examine the dynamics of an induced thrombus in treated and control mice [32]. The vein was then pinched using a standard pressure to introduce injury and to induce a clot. Clot formation and dissolution in the transilluminated vein were visualized with a microscope equipped with closed-circuit video system (including a color monitor and a recorder). When a thrombus reached maximum size, it was measured (in μm2) by digitizing the image and tracing the outer margin of the thrombus. Three to five thrombi were induced in each animal, and the mean thrombus area was computed for each group of animals. The person performing the surgery and measurements (XL) was blinded as to what treatment had been given to each animal.

2.3. In-vitro analysis of cytokine production by splenocytes derived from the APS mice

Spleen cells were isolated from the APS mice, orally given PBS, β2GPI, DI- β2GPI, DV- β2GPI, (n = 10 mice per group). Red blood cells were lysed by using red-blood-cells lysis buffer (Biological Industries Israel Beit-Haemek Ltd). The spleen cells were seeded (5×10^5 cells/well) in 24-well plates (Nunclon™, Roskilde, Denmark), precoated with anti-CD3 Abs (2 μg/ml). PBS, β2GPI, DI- β2GPI, DV- β2GPI were subjected to the cultures at 5 μg/ml in RPMI1640 enriched medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-mercaptoethanol (Biological Industries Beit-Haemek Ltd, Israel). After 72hrs of incubation at 37 °C and 5% CO_2, the supernatants were collected. Cytokine levels of IFNγ, IL-6, IL-17, IL-10, TGFβ in the culture supernatant were detected by DuoSet ELISA kits (R&D systems Minneapolis, MN, USA) according to manufacturer instructions.

2.4. Analysis of T regulatory ($CD4^+CD25^+FOXP3^+$) cells by flow cytometry

Isolated splenocytes form the PBS, β2GPI, DI- β2GPI, DV- β2GPI treated APS mice were depleted of red blood cells. Isolated splenocytes, depleted of red blood cells, followed by blocking Fc receptor with FcBlock. The cells were incubated with anti-$CD4^+$FITC anti-$CD25^+$APC *anti*-$FOXP3^+$PE (eBioscience, San Diego, CA, USA) for Treg detection and analyzed by flow cytometry with forward and side scatter gates adjusted to include all cells and to exclude debris (Becton Dickinson, Franklin Lakes, NY, USA). For intracellular staining of FOXP3, the cells were pre-incubated with a fixation solution, washed, and resuspended in permeabilization solution (Serotec, Oxford, UK) and intracellularly stained for FOXP3. The gating for Tregs was on the $CD4^+$ T cells. The flow cytometry used was from Becton Dickinson, Franklin Lakes, NY, USA.

2.5. Circulating miRNA analyses

Mouse sera were collected from PBS, β2GPI, DI- β2GPI treated groups as a pool, 500 μl) and were used for miRNA extraction using the miRNAeasy mini kit followed by miRNeasy mini kit followed by MinElute Cleanup Kit (QIAGEN Valencia, CA, USA) according the manufacturer's conditions. This procedure was made three times for each sample of mice undergo oral administration of β2GPI, DI- β2GPI, or PBS. The miRNA expression was analyzed by a quantitative real-time PCR. The reactions were carried out on a StepOnePlus™ Real-Time PCR machine, containing 1 Master Mix (Applied Biosystems®), with 1 probes (TaqMan® microRNA Assays, miRNA-155; miRNA-146a; miRNA-182, miRNA-98, (Applied Biosystems®), cDNA sample, and RNU44 endogenous control (Taq-Man microRNA Assay 001094, Applied Biosystems, Foster City,USA) which was used to normalize the results, regarding the biomarkers, since it presents a constant expression level. Data analysis was carried out using the StepOne Software v 2.2 (Applied Biosystems®) with the same baseline and threshold set for each plate, in order to generate threshold cycle (Ct) values for all the miRNAs in each sample. MiRNA quantification was performed in duplicate.

2.6. Statistical analysis

Differences among the studied groups were tested using analysis of variance (ANOVA) according to the data. The analyses that were statistically significant were followed by Tukey's test to identify specific differences between groups. Statistical analysis was performed using SPSS software version 17 (SPSS Inc., Chicago, IL, USA) Results are shown as averages ± standard deviation. Values of $P < 0.05$ were considered significant; P reported values correspond to Tukey's test for specific group differences.

3. Results

3.1. Feeding with D1-β2GPI mitigate manifestations of experimental APS

Experimental APS was induced by immunization with β2GPI affinity purified or with DI- β2GPI. APS mice were fed with domain-I (D1)-β2GPI, domain-V (DV)-β2GPI or β2GPI affinity purified. We analyzed the feeding effect on APS manifestations such as fetal loss and thromboses in the APS fed mice. The mice in the two studied immunization protocols developed experimental APS exhibited by enhanced numbers of fetal loss 39 ± 6 and 48 ± 6 respectively, and reduced number of platelet count 628 ± 101, 504 ± 71 mm^3x10^3 respectively.

Assessing the effect of the compounds on fetal loss revealed, a significant reduction in the percentage of fetal loss in APS mice which were fed with β2GPI, D1- β2GPI (18 ± 3 $p < 0.02$, 6 ± 1 $p < 0.004$ respectively) or fed with DV- β2GPI 37 ± 7 $p > 0.05$, all in

Table 1a
Oral feeding with DI-β2GPI inhibit fetal loss in APS mice.

Immunizing antigen	Oral treatment	% Fetal loss	Platelet count (cells/mm^3 × 10^{-3})
β2GPI n = 60	β2GPI	18 ± 3 P < 0.02	1142 ± 307 p < 0.02
	DI-β2GPI	6 ± 1 p < 0.001	1234 ± 214 P < 0.02
	DV-β2GPI	37 ± 7 p > 0.05	581 ± 89 p > 0.05
	PBS	39 ± 6	628 ± 101
DI-β2GPI n = 60	β2GPI	22 ± 3 P < 0.03	994 ± 112 P < 0.03
	DI-β2GPI	7 ± 0.8 P < 0.001	1242 ± 254 P < 0.02
	DV-β2GPI	49 ± 4 p > 0.05	632 ± 78 p > 0.05
	PBS	48 ± 6	504 ± 71

comparison to APS mice fed with PBS 39 ± 6, (Table 1a).

Feeding APS mice with β2GPI or D1- β2GPI, attenuated thrombus formation *ex-vivo*. IgG from mice fed with β2GPI or D1- β2GPI that was passively transferred to naïve mice had significantly lower thrombi size when compared to IgG purified from mice fed with PBS (1145 ± 276 μm^2 p < 0.04, 224 ± 67 μm^2 p < 0.001 respectively), whereas, IgG from mice fed with DV- β2GPI did not inhibit thrombus size 2851 ± 304 μm^2 p > 0.05, all compared to IgG from PBS fed mice which resulted in clot size of size 3765 ± 465 μm^2 (Table1b).

3.2. D1- β2GPI feeding alleviate anti -β2GPI production by APS mice

Naive mice immunized with β2GPI whole molecule, developed experimental APS. Six weeks later, anti- β2GPI Abs titers were elevated and reached plateau. The APS mice were fed with β2GPI whole molecule, or recombinant DI or derivative of DV or PBS. Seven weeks later the effect of the oral treatments was analyzed. Feeding with β2GPI and D1- β2GPI, diminished generation of *anti*-β2GPI antibodies OD 0.82 ± 0.07 (p < 0.01) and *anti*-D1- β2GPI OD 0.38 ± 0.04 (p < 0.0001), whereas feeding of APS mice with DV showed a weak significance in reduction of anti- β2GPI Abs titer (p < 0.04), all in comparison to APS mice fed with PBS, as illustrated in Fig. 1a.

Naïve mice immunized with D1- β2GPI, developed anti- β2GPI Abs and fed with β2GPI whole molecule, or recombinant DI- β2GPI or derivative of DV- β2GPI or PBS. As demonstrated in Fig. 1b, the D1- β2GPI immunized mice developed elevated titers of anti- β2GPI Abs (OD 1.45 ± 0.26), and *anti*-D1-β2GPI (OD 1.37 ± 0.31) p > 0.05 and low titers of *anti*-DV- β2GPI Abs (0.45 + 0.07 p < 0.002) in comparison to PBS treated mice (OD 1.45 ± 0.18). The fed mice with β2GPI or D1-β2GPI developed a significant lower titers of anti- β2GPI or *anti*-D1-β2GPI (p < 0.001,p < 0.002 respectively) compared with titers of *anti*-β2GPI and *anti*-D1-β2GPI before the feeding respectively. Whereas, mice immunized with D1-β2GPI and fed with DV- β2GPI, developed low titers of *anti*-DV-β2GPI Abs (p > 0.05) in comparison to *anti*-DV-β2GPI before feeding.

Table 1b
Oral feeding with DI-β2GPI inhibit thrombus size formation.

Oral treatment	Thrombus size μm^2
β2GPI n = 40	1145 ± 276 p < 0.01
DI-β2GPI n = 40	224 ± 67 P < 0.003
DV-β2GPI n = 40	2851 ± 304 p > 0.05
PBS n = 40	3002 ± 276

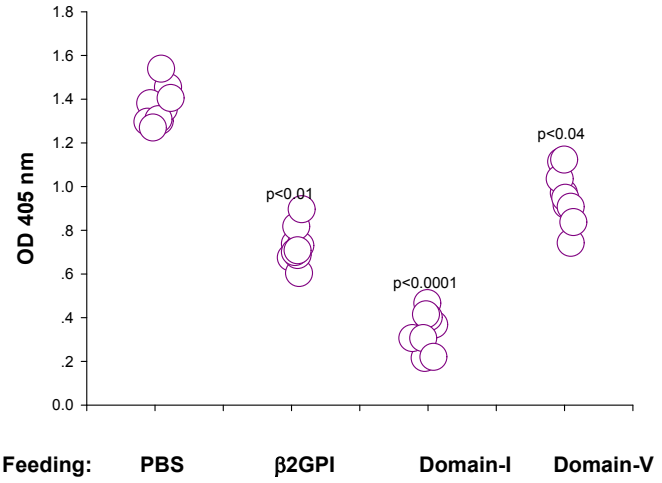

Fig. 1. a: APS mice induced by immunization with β2GPI and fed with PBS, β2GPI, DI- β2GPI, DV- β2GPI separately. Generation of anti- β2GPI was tested in all the studied groups of mice. Antibodies to different domains of β2GPI were analyzed. The data are presented as mean ± SD of OD at 405 nm, of 3 separate experiments, n = 60. b: APS mice induced by DI- β2GPI immunization with β2GPI and fed with PBS, β2GPI, DI- β2GPI or DV- β2GPI. The data presented show anti- β2GPI titers of antibodies directed to different domains before and after oral ingestion. The data are presented as mean ± SD of OD at 405 nm, of 3 separate experiments, n = 60.

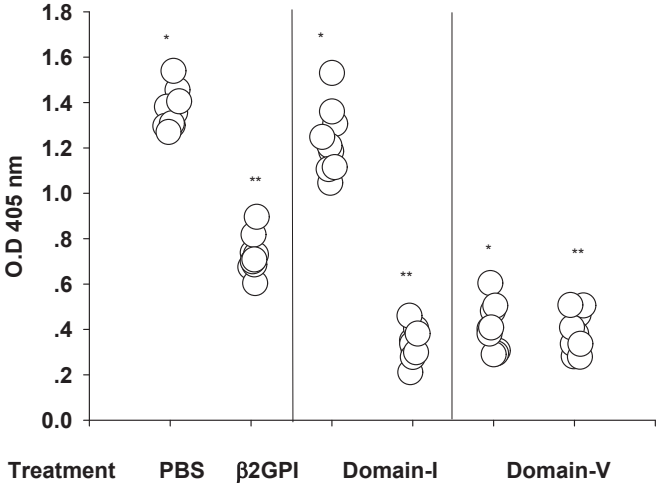

Fig. 1. (*continued*)

3.3. APS mice fed with D1- β2GPI showed attenuation of inflammatory cytokines production

APS mice induced by immunization with β2GPI, fed with D1- β2GPI or β2GPI whole molecule, showed a significant decrease in expression of inflammatory cytokines: IFNγ (643 ± 19 pg/ml, p < 0.001) and IL-17 (703 ± 98 pg/ml, p < 0.001) by splenocytes, compared to PBS subjected group of APS mice (1254 ± 162 pg/ml, 1354 ± 112 pg/ml respectively), as shown in Fig. 2. No inhibition in IL-17 production was shown in APS mice fed with DV- β2GPI (p > 0.05). Likewise, a weak but a significant decreased production of IFNγ was noticed in the APS mice treated with DV- β2GPI (p < 0.04). IL-6 secretion by splenocytes *in-vitro* upon feeding with D1- β2GPI or β2GPI whole molecule (101 ± 22 pg/ml, 142 ± 19 pg/ml, in comparison to 862 ± 112 pg/ml in APS mice fed with DV- β2GPI or with PBS 704 ± 32 pg/ml) (p < 0.001).

Moreover, the cells showed enhanced expression of anti-inflammatory cytokines IL-10 (879 ± 104 pg/ml) and TGFβ (765 ± 97 pg/ml) upon feeding of APS mice with D1- β2GPI, in

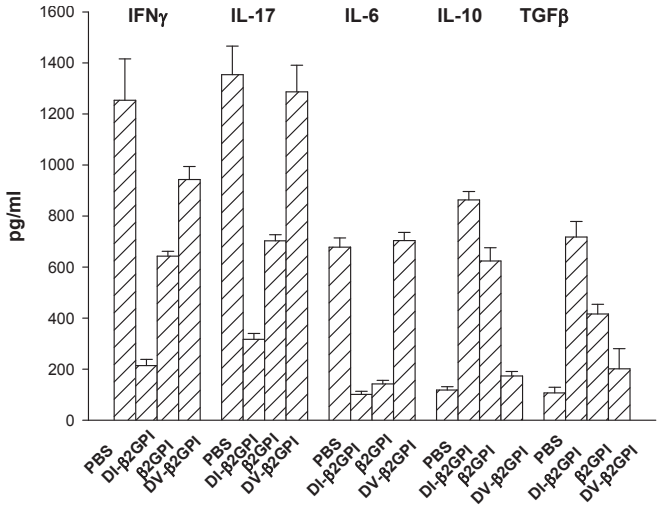

Fig. 2. The effect of oral tolerance with β2GPI on cytokines expression. The data show for each cytokine concentration, produced *in-vitro* by splenocytes from the APS treated mice, fed with PBS, or DI-β2GPI, β2GPI or DV- β2GPI. The data are presented as pg/ml, of 3 separate experiments, n = 30.

comparison with PBS fed mice, ($p < 0.002$) as illustrated in Fig. 2a. Feeding with β2GPI, enhanced the IL-10 and TGFβ production as well ($p < 0.03$, $p < 0.02$ respectively), compared to feeding with PBS. APS mice fed with DV- β2GPI showed a decrease in IFNγ production ($p < 0.04$), but no effect on IL-17 or IL-10, or TGFβ production ($p > 0.05$).

3.4. D1- β2GPI feeding of APS mice resulted in an expansion of T regulatory cells

Elevated number of $CD4^+CD25^+FOXP3^+$ T regulatory cells (Tregs) were documented in the spleen of the APS mice induced by β2GPI and fed with D1- β2GPI, as illustrated in Fig. 3. The cells were analyzed by FACS, gating on $CD4^+$ cells and counting the CD25/FOXP3 cells, Fig. 3. The percent of Tregs was elevated from 2.58% to 5.6%, by 2.2, $p < 0.04$ in comparison with the expansion of the PBS fed APS mice. No enhanced expansion in Treg cells was noticed in the DV- β2GPI.

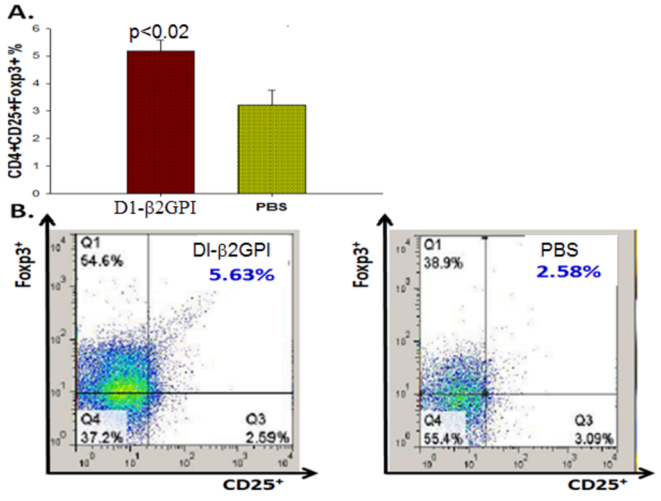

Fig. 3. T-regulatory cells in APS mouse splenocytes treated with DI-β2GPI. $CD4^+CD25$ + FOXPE + Treg cells in splenocytes from DI-β2GPI fed mice in comparison to PBS ingested mice. Data are presented by FACS and percentage of Tregs, n = 30.

3.5. Differential expression of miRNAs related to tolerance induction with D1- β2GPI fed APS mice

miRNA was analyzed in the sera of the APS mice induced by β2GPI and fed with D1- β2GPI. Enhance expression of circulating miRNA-155 ($p < 0.001$), miRNA-182 ($p < 0.03$), miRNA-98 ($p < 0.01$), and a weak significance in attenuation of miRNA146a ($p < 0.048$), all in comparison to naïve mice before disease induction. The D1- â2GPI fed APS mice had a reduced expression of circulating miRNA-155 ($p < 001$), miRNA-181a ($p < 0.02$), miRNA-98 ($p < 0.02$) and enhanced circulating miRNA-146a ($p < 0.04$), as described in Fig. 4.

4. Discussion

The APS disease is characterized by recurrent fetal loss, repeated thromboembolic phenomena, thrombocytopenia, and prolonged aPTT measurements. These diverse clinical pictures are associated with elevated titers of mainly circulating antiphospholipid β-2-glycoprotein-I (β2GPI)-dependent Abs, *anti*-β2GPI Abs [1–6]. Circulating *anti*-β2GPI Abs target diverse epitopes on the five domains of the β2GPI molecule [8–11]. Groot PG et all [12,13] proved that the circular β2GPI is opened when β2GPI target a membrane structure, than, domain-I the cryptic immunogenic epitope is exposed to the immune system to which anti-domain-I Abs are generated. Therefore, APS patients develop mainly *anti*-β2GPI antibodies targeting domain-I and less domain-V of the β2GPI. During the last decades vast publications pointed to the strong association with APS manifestation such as fetal loss [21,23,27] and thrombosis [14–16,25,27–29]with circulating elevated titers of antibodies targeting DI-β2GPI. Although, one study did not show a direct thrombotic effect of *anti*-DI-β2GPI in a rat model of thrombosis [26].

Previously we have demonstrated that β2GPI fed APS mice induced by immunization with β2GPI, developed tolerance exemplified by reduction in the classical experimental APS manifestations; e.g. enhanced number of fetal loss, thrombocyte count and higher APTT values [31]. Recently, we had shown that tolerogenic dendritic cells loaded with domain-I, attenuated experimental antiphospholipid syndrome [21]. In the current study we assessed the posibility that oral treatment with DI-β2GPI may be a tool for induction of tolerance in experimental APS mice.

Analyzing the domain specific antibodies, we can see that in mice immunized with the β2GPI whole molecule developed antibodies directed differentially to the whole molecule. Mice immunized with DI-β2GPI developed *anti*-β2GPI and also low levels of anti- DV-β2GPI. Previously we have demonstrated that immunization with human β2GPI developed during the time antibodies to mouse β2GPI due to similarity between human and mouse β2GPI [45]. There is 76% identity and 86% similarity when comparing accession number AAB21330.1 of human β2GPI to accession number of CAA69401 of mouse β2GPI, in the protein database. This may explain the low level raise of anti- DV-β2GPI antibodies as a result of DI-β2GPI immunization and the epitope spread phenomenon. Since the *anti*-DV-β2GPI levels were very low, feeding with DV-β2GPI did not have any significant effect on the *anti*-DV-β2GPI levels.

In addition, as presented in the current results, β2GPI (whole molecule) was less effective than DI-β2GPI in the induction of oral tolerance. This result may lead to the idea that the response to DI-β2GPI was stronger, because it's the exposed sequence of the β2GPI hidden epitope [12.13], than to the 6 domains. Therefore feeding with DI-β2GPI was significantly more impressive in the antibodies response.

Our data reveal that experimental APS induced by DI-β2GPI or β2GPI, fed with DI-β2GPI or β2GPI induced tolerance exemplified by reduced generation of *anti*-β2GPI Abs. APS tolerized mice with DI-β2GPI developed enhanced Treg splenic population. The DI-β2GPI fed APS mice had lower prevalence of inflammatory cytokines such as IFNγ, IL-6 and IL-17, whereas enhanced expression of anti-inflammatory IL-

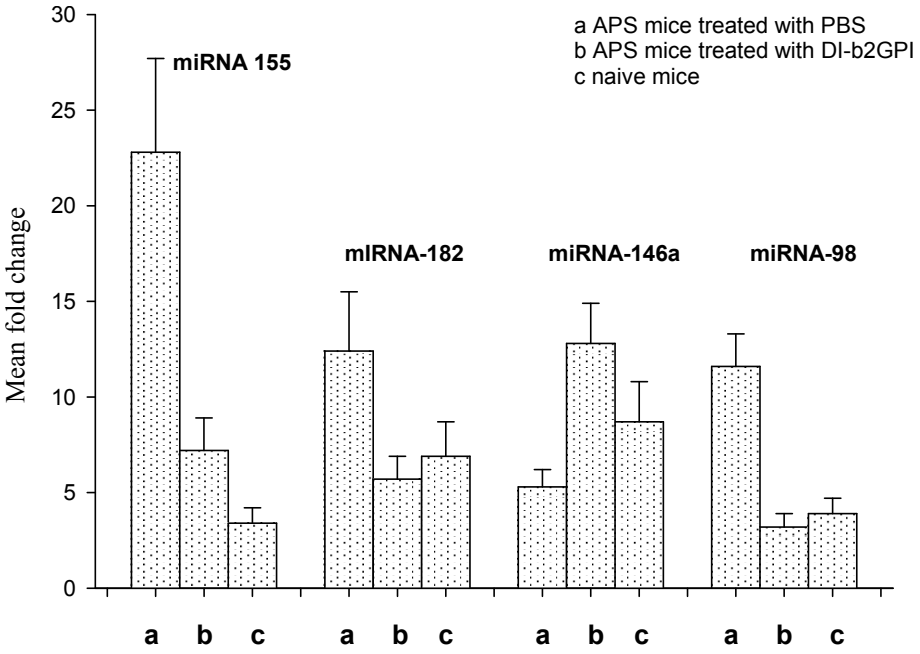

Fig. 4. miRNA in the sera of the DI- β2GPI tolerized mice. Circulating miRNA-155, miRNA-146a, miRNA-182, and miRNA-98, expression in the sera of APS mice tolerized mice with DI- β2GPI. The RT-PCR data are presented as mean fold change of 3 separate expreriments n = 60.

10 and TGFβ.

Likewise, the tolerogenic makeup in the DI-β2GPI fed APS mice was associated with immunomodulating circulatory miRNAs.

miRNAs, are a group of small non-coding RNA, comprised of approximately 18–25 nucleotides, miRNAs regulates post transcriptional gene expression mostly by degradation or translation inhibition of targeted mRNAs [31,33,34]. MiRNA are important in regulating immune cell differentiation and immune responses including inflammation, autoimmunity and cancer [31,33–36]. Diverse miRNAs are associated with autoimmune disease activity and clusters of miRNA are served as biomarkers of a disease, such as in systemic lupus erythematosus (SLE) [35–37], rheumatoid arthritis (RA) [38,39] and antiphosphlipid syndrome [40–42]. In our APS experimental model, the feeding with D1-β2GPI and the consequent tolerance induction, resulted in decreased circulating miRNA-155. MicroRNA-mediated regulation of T helper type 17/regulatory T-cell balance in autoimmune disease [43]. Increased expression of circulating miRNA 155 is associated with immune network regulation [43]. Previously we have shown that decreased expression of miRNA-155 is related to tolerogenic dendritic cell phenotype expansion [21]. Furthermore, miRNA-146a was decreased in the APS mice, which is known to negatively regulate NFkB activation, and increase IL-17 expression. Whereas, in the D1-β2GPI tolerogenic mice the circulating miRNA 146a expression is enhanced ($p < 0.01$), compared to circulating miRNA 146a in the APS mice. Likewise, circulating miRNA-182 expression is enhanced in the APS mice and is related to enhanced antibody secretion, NFkB and Th1/Th2 cell activation [36–38]. D1-β2GPI oral tolerance in APS mice resulted in inhibition of circulating miRNA-182 expression ($p < 0.006$). Elevated circulating miRNA-98 expression was observed in APS mice, whereas in the D1-β2GPI fed APS mice it was downregulated ($p < 0.003$). MiRNA-98 was shown to negatively regulate IL10 anti-inflammatory cytokine production by macrophages [41–44].

In conclusion, as *anti*-DI-β2GPI are pathogenic autoantibodies in patients with APS, we assessed the potential of DI-β2GPI derivative to cause oral tolerance in APS mice induced with β2GPI. Our results show that DI-β2GPI fed APS mice developed tolerance scenario manifested by reduction in fetal loss, thrombi size, inhibition of inflammatory cytokine production and upregulation of IL-10 and T regulatory cells. This sequence of events is associated with circulating plasma miRNA related to immune network. Therefore we propose the DI-β2GPI derivative a treatment for patients with APS.

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