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Ann Rheum Dis published online October 21, 2010
doi: 10.1136/ard.2010.137281

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Anti- β_2 -glycoprotein I IgG antibodies from 1-year-old healthy children born to mothers with systemic autoimmune diseases preferentially target domain 4/5: might it be the reason for their 'innocent' profile?

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Accepted 15 August 2010

ABSTRACT

Background Anti- β_2 -glycoprotein-I (anti- β_2 GPI) were demonstrated to be pathogenic in the antiphospholipid syndrome (APS). However, they can be detected in patients with no features of APS, especially those affected by systemic autoimmune diseases (SAD), and so in healthy children. It has been suggested that anti- β_2 GPI against domain 1 (D1) associate with thrombosis, while those recognising domain 4/5 (D4/5) are present in non-thrombotic conditions.

Objective To evaluate the fine specificity of anti- β_2 GPI in adults and infants.

Methods Three groups were examined—group A: 57 1-year-old healthy children born to mothers with SAD; group B: 33 children with atopic dermatitis; group C: 64 patients with APS. Subjects were selected based on positive anti- β_2 GPI IgG results. Serum samples were tested for anti- β_2 GPI IgG D1 and D4/5 using research ELISAs containing recombinant β_2 GPI domain antigens.

Results Children (A and B) displayed preferential IgG reactivity for D4/5, whereas patients with APS were mainly positive for D1. No thrombotic events were recorded in groups A and B.

Conclusions The specificity for D4/5 suggests that anti- β_2 GPI IgG production in children born to mothers with SAD is a process neither linked to systemic autoimmunity nor related to the maternal autoantibody status. This unusual fine specificity might, at least partially, account for the 'innocent' profile of such antibodies.

INTRODUCTION

Anti- β_2 -glycoprotein I antibodies (anti- β_2 GPI) are a formal laboratory criterion for the antiphospholipid syndrome (APS)¹ and were shown to be pathogenic for both APS-related thrombosis and pregnancy morbidity.² Anti- β_2 GPI, however, can be detected in individuals with no clinical events related to APS.

It is possible to find anti- β_2 GPI in patients with systemic autoimmune diseases (SAD), but few of them develop APS. Factors that contribute to, or prevent, the development of APS are multiple, and their interaction may be different from case to case.³

Anti- β_2 GPI have also been described in a wide range of non-autoimmune conditions such as atherosclerotic syndrome⁴ and leprosy,⁵ and in children with atopic dermatitis (AD).⁶ Even apparently

healthy children, seen at regular preventive visits, may also carry anti- β_2 GPI.⁷ Our group reported that nearly 50% of 1-year-old healthy children born to mothers with SAD may test positive for anti- β_2 GPI.⁸

The association of anti- β_2 GPI with a spectrum of conditions spanning healthy children to those with typical APS may be partly explained by the variability in sensitivity and specificity of the assays, with a different threshold for positivity.⁹ However, the existence of confirmed, medium-high titre anti- β_2 GPI in non-APS conditions has also raised the hypothesis that 'pathogenic' antibodies may have a different specificity from 'innocent' ones.

β_2 GPI is a single-chain protein composed of five repeating sequences ('domains'). Domain 5 (D5) is critical for binding to anionic phospholipid membranes, while domain 1 (D1) projects into the extracellular space and can interact with other proteins/antibodies.¹⁰ Preliminary studies showed that anti- β_2 GPI from patients with APS have a preferential recognition for D1.¹¹ Anti-D1 antibodies seem to best correlate with a history of thrombosis,¹² so they have been claimed to be the specific anti- β_2 GPI subgroup associated with APS. Additionally, an experimental animal model demonstrated that the thrombotic effect of antiphospholipid antibodies could be reduced using a peptide that mimics D1.¹³

Specificity for the other end of the molecule was shown in non-thrombotic conditions. Anti- β_2 GPI from atherosclerotic patients⁴ were able to bind to domain 4, while antibodies from patients with leprosy⁵ and children with AD⁶ recognised D5.

With this background, we were prompted to test the fine specificity of anti- β_2 GPI in children born to mothers with SAD, using as control groups children with AD and patients with APS.

PATIENTS AND METHODS

Patients

Subjects were selected based on a positive anti- β_2 GPI IgG antibody result obtained with our routine test.

Three groups were considered:

- Group A: 57 1-year-old healthy children born to mothers with various SAD, who were managed by a multidisciplinary team during pregnancy and whose children were usually

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seen by dedicated neonatologists. Informed consent was received from the parents, and their infants were tested at birth for the evaluation of the transplacental passage of maternal autoantibodies. They were retested at 1 year to check autoantibody clearance. Fourteen mothers (25%) displayed anti- β_2 GPI IgG before and during pregnancy; their children did not have significantly different titres from those of children born to anti- β_2 GPI IgG negative mothers.

- ▶ Group B: 33 children with AD observed at the Children's Hospital in Ljubljana.
- ▶ Group C: 64 patients with definite APS, according to the revised criteria.¹ Twenty-eight (43.8%) had arterial and/or venous thrombosis, 24 (37.5%) obstetrical complications and 12 (18.8%) both manifestations. No thrombotic events were recorded in either group A or B. The study was approved by the local ethical committees.

Methods

Anti- β_2 GPI IgG were tested by a validated home-made ELISA assay, routinely performed in our laboratory.¹⁴ Results are expressed as optical density (OD) values.

The fine specificity of these antibodies was investigated using prototype research ELISAs developed by INOVA Diagnostics (San Diego, California, USA). Two different portions of β_2 GPI were separately analysed: D1 and domain 4/5 (D4/5). Briefly, recombinant D1 or D4/5 antigens were coated on ELISA plates, and subsequent incubations with diluted samples, anti-IgG conjugate, tetramethylbenzidine chromogen and stop solution were performed according to the manufacturer's instructions, using the reagents provided in the kit. Controls were available in the anti-D1 IgG kit, whereas a patient sample strongly positive for anti-D4/5 IgG was used as a D4/5 control on each run at serial dilutions. Results were expressed as OD values. One hundred adult normal healthy donors were also tested for calculation of the cut-off point, which was set at the 95th centile (0.235 OD for anti-D1 IgG and 0.405 OD for anti-D4/5 IgG).

Statistical analysis

Antibody titres were compared using the Mann-Whitney test; a p value <0.05 was considered significant. For linear regression, an r value between 0.500 and 0.700 was considered as moderate correlation, r>0.700 as good correlation.

RESULTS

As shown in table 1, anti- β_2 GPI IgG from subjects in groups A and B displayed a preferential recognition for D4/5, while there is a predominance of anti-D1 in group C.

Table 1 Different patterns of positivity for IgG anti-D1 and IgG anti-D4/5 in each group

	D1+ D4/5- n (%)	D1- D4/5+ n (%)	D1+ D4/5+ n (%)	D1- D4/5- n (%)
One-year-old children (n=57)	4 (7)	16 (28)	5 (8.8)	32 (56.2)
Children with AD (n=33)	7 (21.2)	9 (27.3)	2 (6.1)	15 (45.5)
APS (n=64)	43 (67.2)	3 (4.7)	5 (7.8)	13 (20.3)

Results are shown as number (%).

Positive results were identified according to the cut-off point calculated on 100 normal healthy donors (see the 'Methods' section)

AD, autoimmune disease; APS, antiphospholipid syndrome; D1, domain 1; D4/5, domain 4/5.

Anti- β_2 GPI IgG values ranged from low to high positive in all groups with a similar distribution, while there was a significant difference between titres of anti-D1 and anti-D4/5 in each group (see figure 1).

Taking into consideration such a polarisation, we looked for a correlation between anti- β_2 GPI IgG and anti-D4/5 in group A and B (r=0.802, r=0.879), and between anti- β_2 GPI IgG and anti-D1 in group C (r=0.579). The p values for all correlations were <0.05.

DISCUSSION

The interpretation of a positive result for antiphospholipid antibodies remains a difficult task, particularly in those patients who do not fulfil APS clinical criteria, since the approach to such cases is much less formalised.

Detection of anti- β_2 GPI in healthy children⁷ and in children without APS-related disease such as AD⁶ raises the possibility of the existence of 'innocent' anti- β_2 GPI. Our own group has also observed the presence of anti- β_2 GPI in 1-year-old healthy children born to mothers with SAD.⁸ Interestingly, most of these women were negative for anti- β_2 GPI before and during pregnancy. Thus, the detection of anti- β_2 GPI suggests a de novo production of antibodies, not related to the maternal background, but rather to environmental factors acting on the children's immune system. β_2 GPI is a highly conserved protein and is ubiquitous in food. Therefore, it is possible that the ingestion of bovine or other types of β_2 GPI could induce a per oral immunisation and induce transitory production of anti- β_2 GPI in infants. In addition, exposure to viral infections and vaccinations, which are frequent in the first years of life, might induce both a specific and a non-specific immune response, including anti- β_2 GPI production.^{7 8}

Prompted by the literature reporting that different subpopulations of anti- β_2 GPI may be associated with either APS or non-thrombotic, non-autoimmune conditions,^{4-6 11 12} we used research ELISA methods for the investigation of anti-D1 and anti-D4/5-specific antibodies.

In children (both group A and B) we observed a predominance of anti-D4/5, which were present in nearly 30% of the subjects. Titres of anti-D4/5 were also significantly higher than those of anti-D1 in both groups. However, it should be noted that nearly half of the subjects were negative for both anti-D1 and anti-D4/5, probably owing to the use of a cut-off point which was based on an adult population.

Turning to group C, the control group of patients with APS, we found that anti-D1 are the most prevalent antibodies (67%). This is consistent with a recent international multicentre study that showed anti-D1 in 55% of the patients.¹⁵ In contrast, isolated positivity for anti-D4/5 was rarely seen. We identified three patients with APS, all with a history of fetal losses, but no thrombotic events. It should be pointed out that 20% of our group C cohort was negative for both anti-D1 and anti-D4/5. This should not be surprising if we consider that antibodies to different portions of the β_2 GPI could exist.^{11 16} This observation should make us aware that an APS-related event may occur despite the absence of any detectable anti-D1. In fact, a negative result for anti-D1 may be greatly affected by the sensitivity of the method; on the other hand, an APS-related event is determined by several factors, among which, but not exclusively, anti-D1 antibodies.

In conclusion, this study demonstrated for the first time that anti- β_2 GPI IgG detected in 1-year-old children preferentially

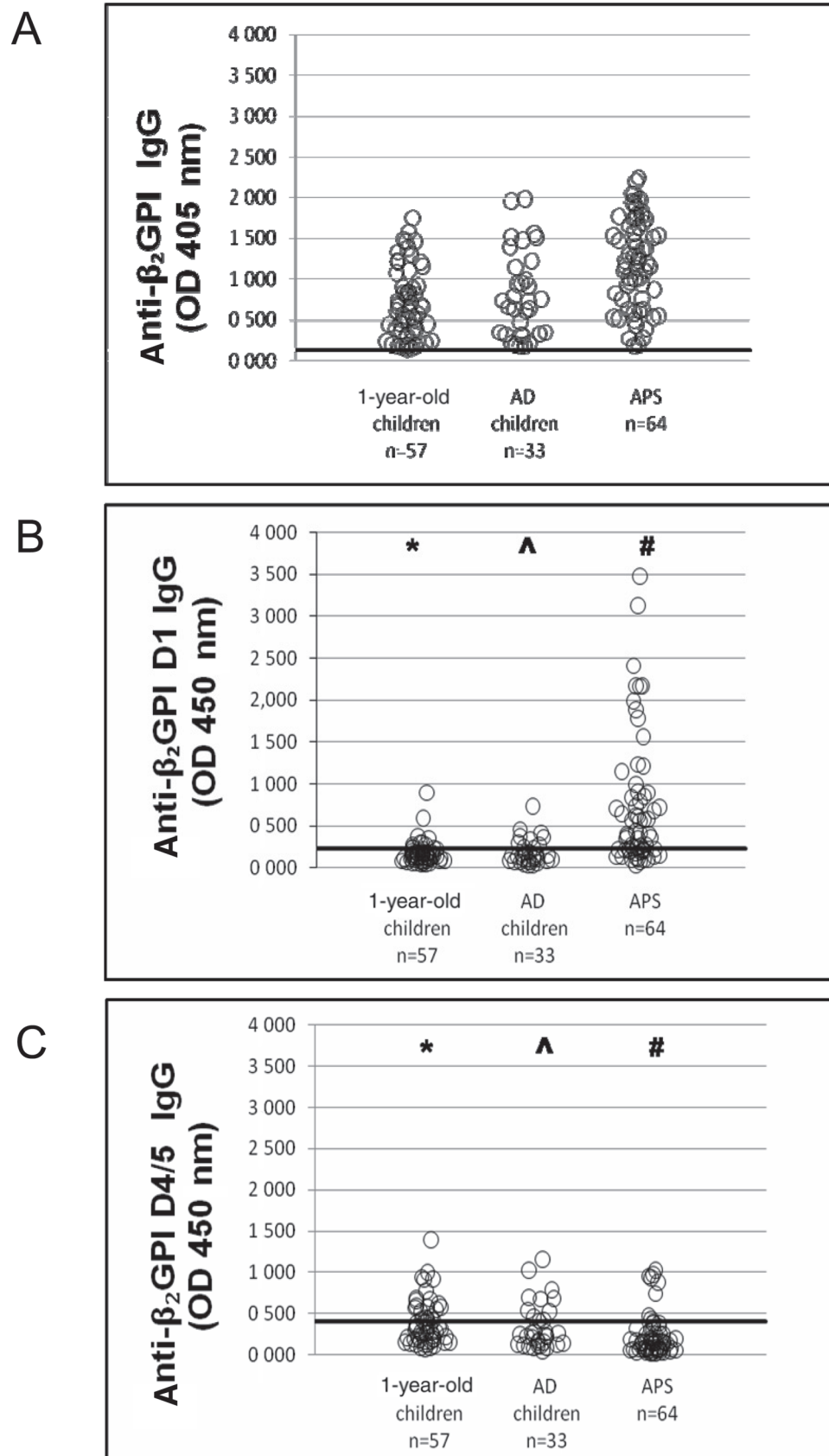


Figure 1 Distribution of anti- β_2 -glycoprotein-I (anti- β_2 GPI) IgG (A), anti-domain 1 IgG (D1) (B) and anti-domain 4/5 IgG (D4/5) (C) in the three groups. One-year-old children: group A; 1-year-old children born to mothers with systemic autoimmune diseases (AD children): group B; children with atopic dermatitis (APS): group C, patients with antiphospholipid syndrome. Comparison of anti-D1 IgG and anti-D4/5 IgG within each group was performed using the Mann–Whitney test. Group A: * $p < 0.0001$, group B: ^ $p = 0.03$, group C: # $p < 0.0001$. The bold line represents the cut-off point for each test.

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recognise D4/5. We were able to confirm previous data on children with AD,⁶ who also have antibodies to D4/5. In contrast, we found a predominance of anti-D1 in patients with APS. It would be tempting to speculate that anti-D1 are the pathogenic antibodies, whereas anti-D4/5 are typical of non-autoimmune conditions and thus possibly 'innocent'. Although isolated anti-D4/5 have been found in APS, they are rare and not associated with thrombosis. In our opinion this does not allow us to draw definite conclusions about the possible 'mild' pathogenic potential of anti-D4/5. It should be examined whether, in the presence of a proper 'second hit',¹⁷ anti-D4/5 could be 'activated' and mediate the disease mechanism in a manner similar to anti-D1. In fact, one may consider that children are an unusual model in which no additional cardiovascular risk factor is present, so anti- β_2 GPI are less likely to be 'stimulated'. In such a view, it could be helpful to extend the investigation on anti-domain antibodies to non-APS systemic autoimmune conditions, in order to determine if the absence of APS manifestations might be associated with the absence of antibodies to a particular anti- β_2 GPI domain, in particular, the absence of anti-D1.

Acknowledgements Thanks to all the parents for allowing their children to be included in the study and to Dr Filippo Sarra (IL, Milan, Italy) for allowing prompt delivery of the prototype anti-domain kits.

Competing interests GLN, ZS, SE and WLB are employees of INOVA Diagnostics Inc.

Ethics approval This study was conducted with the approval of the local ethics committees.

Provenance and peer review Not commissioned; externally peer reviewed.

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