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New insight into antiphospholipid syndrome: antibodies to β2glycoprotein I-domain 5 fail to induce thrombi in rats

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ARD

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?

Laura Andreoli, Cecilia Nalli, Mario Motta, Gary L Norman, Zakera Shums, Susan Encabo, Walter L Binder, Monica Nuzzo, Micol Frassi, Andrea Lojacono, Tadej Avcin, Pier-Luigi Meroni and Angela Tincani

Ann Rheum Dis 2011 70: 380-383 originally published online October 21, 2010

ARTHRITIS & RHEUMATOLOGY
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DOI 10.1002/art.39187

Clinical Characterization of Antiphospholipid Syndrome by Detection of IgG Antibodies Against β_2-Glycoprotein I Domain 1 and Domain 4/5

Ratio of Anti–Domain 1 to Anti–Domain 4/5 As a Useful New Biomarker for Antiphospholipid Syndrome

Laura Andreoli,[1] Cecilia B. Chighizola,[2] Cecilia Nalli,[1] Maria Gerosa,[3] M. Orietta Borghi,[2] Francesca Pregnolato,[4] Claudia Grossi,[4] Alessandra Zanola,[1] Flavio Allegri,[1] Gary L. Norman,[5] Michael Mahler,[5] Pier Luigi Meroni,[2] and Angela Tincani[1]

Journal of Autoimmunity 90 (2018) 76-83
Beyond thrombosis: Anti-β2GPI domain 1 antibodies identify late pregnancy morbidity in anti-phospholipid syndrome
CB Chighizola et al.

In APS serum samples:

- many observational clinical studies confirmed the presence of different domain specific anti-β2 antibodies
- main reactivity against D1 (IgG aD1)
- reactivity against D4/5 observed, lower titers and frequency (IgG aD4/5)
- no significant reactivity against D2 and D3 (high glycosylation sites)

… **BUT**.. If nowadays aD1 have a known pathogenic role…

… What do these aD4/5 represent?

… Why are they found both in symptomatic and in asymptomatic aPL+ subjects?

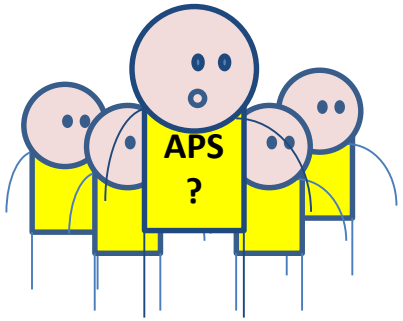
… Have they got a pathogenic role?

… Let me dare.. and if they are not only less or not pathogenic..but even able to dampen aD1 negative effect?**... A kind of «protective role»?...**

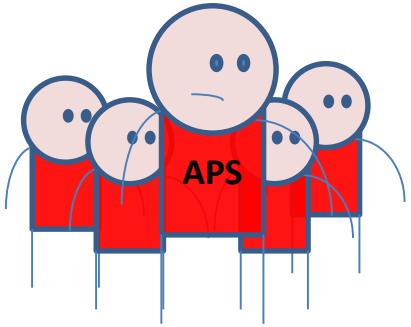
We decided to better investigate aD4/5 role in *«in vivo and in vitro »* APS models

Samples

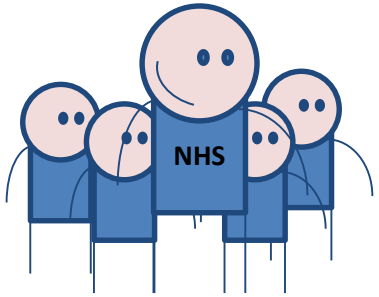
Serum IgG from 3 groups of subjects:



aβ2(whole molecule) pos β2-dependent-aCardiolipin neg LAC neg	**aD4/5 isolated pos**	**Doubtful thrombotic and/or obstetric clinical events**

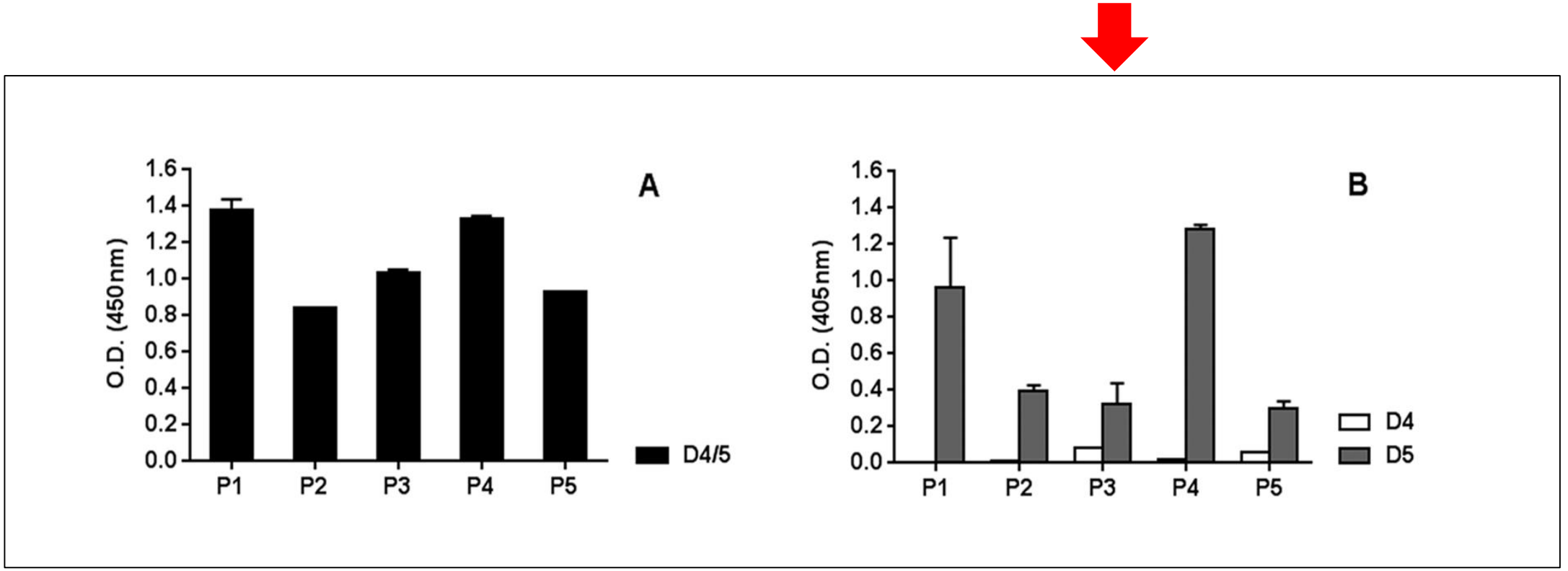


aβ2(whole molecule) pos **β2-dependent-aCardiolipin pos** **LAC pos**	**aD1 isolated pos**	**Clear thrombotic and/or obstetric clinical events**



Healthy Controls

… aD4/5… BUT «these data will talk» about aD5.. WHY??...



EXPERIMENTAL DESIGN (1):

In vivo/ex vivo:

- **setting up of a first in vivo APS model** of thrombosis in rat to evaluate the aD5 or aD1 thrombogenic effect
- **setting up of a second in vivo APS model** in rat to evaluate if aD5 or aD1 could modulate β2GPI deposition on vessels in a different way
- **ex vivo immunofluorescence** analysis to investigate the reactivity of the IgG fractions on the endothelium and β2GPI deposition on the vessels

Aim:

- to answer in particular to these questions:

  1)" **Do aD5 serum IgG have a thrombogenic effect? "**

  2)"**Could aD5 modulate β2GPI binding to the endothelium?**"

EXPERIMENTAL DESIGN (2):

In vitro:

- **setting up of a β2GPI dependent-anti Cardiolipin** assay making serum IgG to interact with β2GPI bound to Cardiolipin
- **setting up of an inhibition assay** making serum IgG to interact with β2GPI in the fluid phase

Aim:

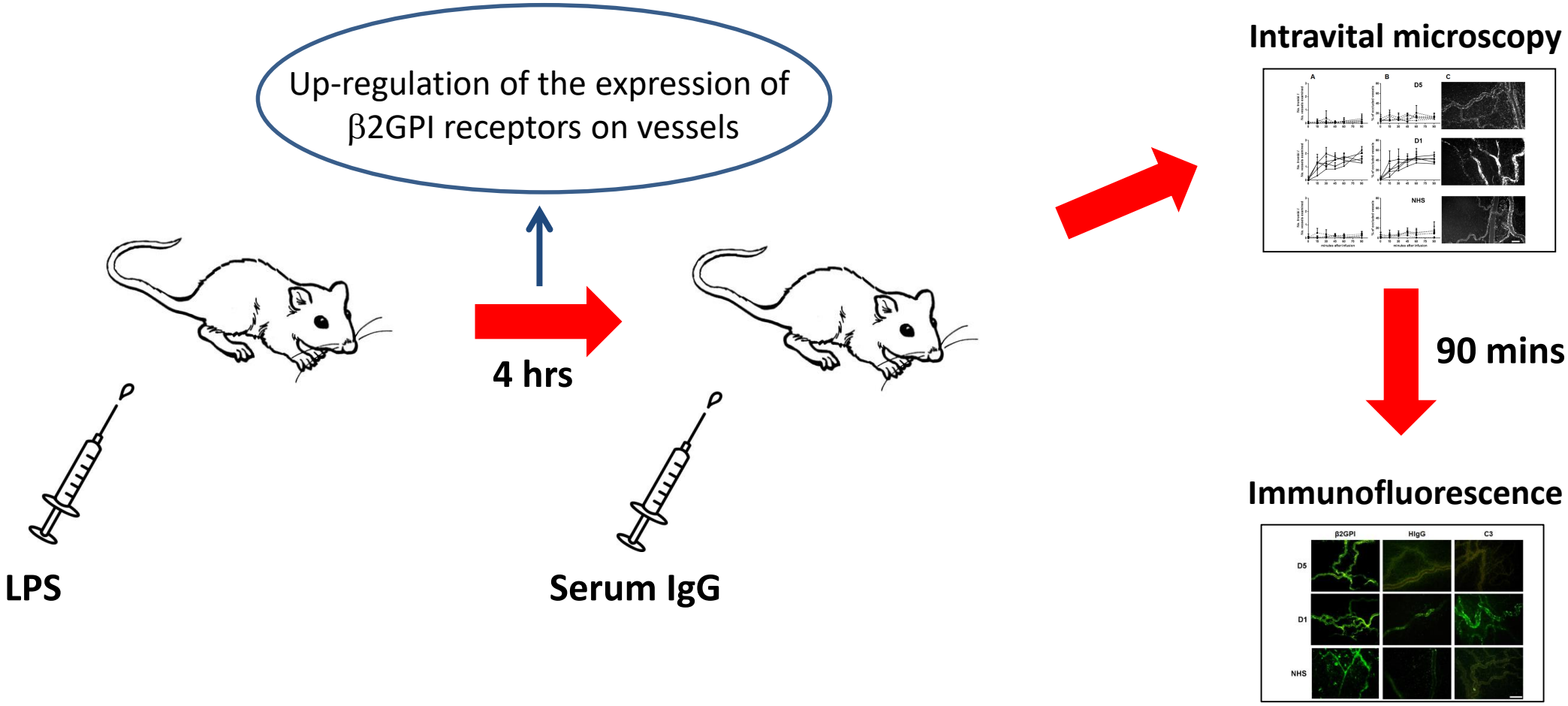
- to answer in particular to these questions:

  1) "**Do aD5 serum IgG interact with β2GPI bound to the phospholipid?"**

  2) "**Do aD5 serum IgG interact with β2GPI in the fluid phase?** "

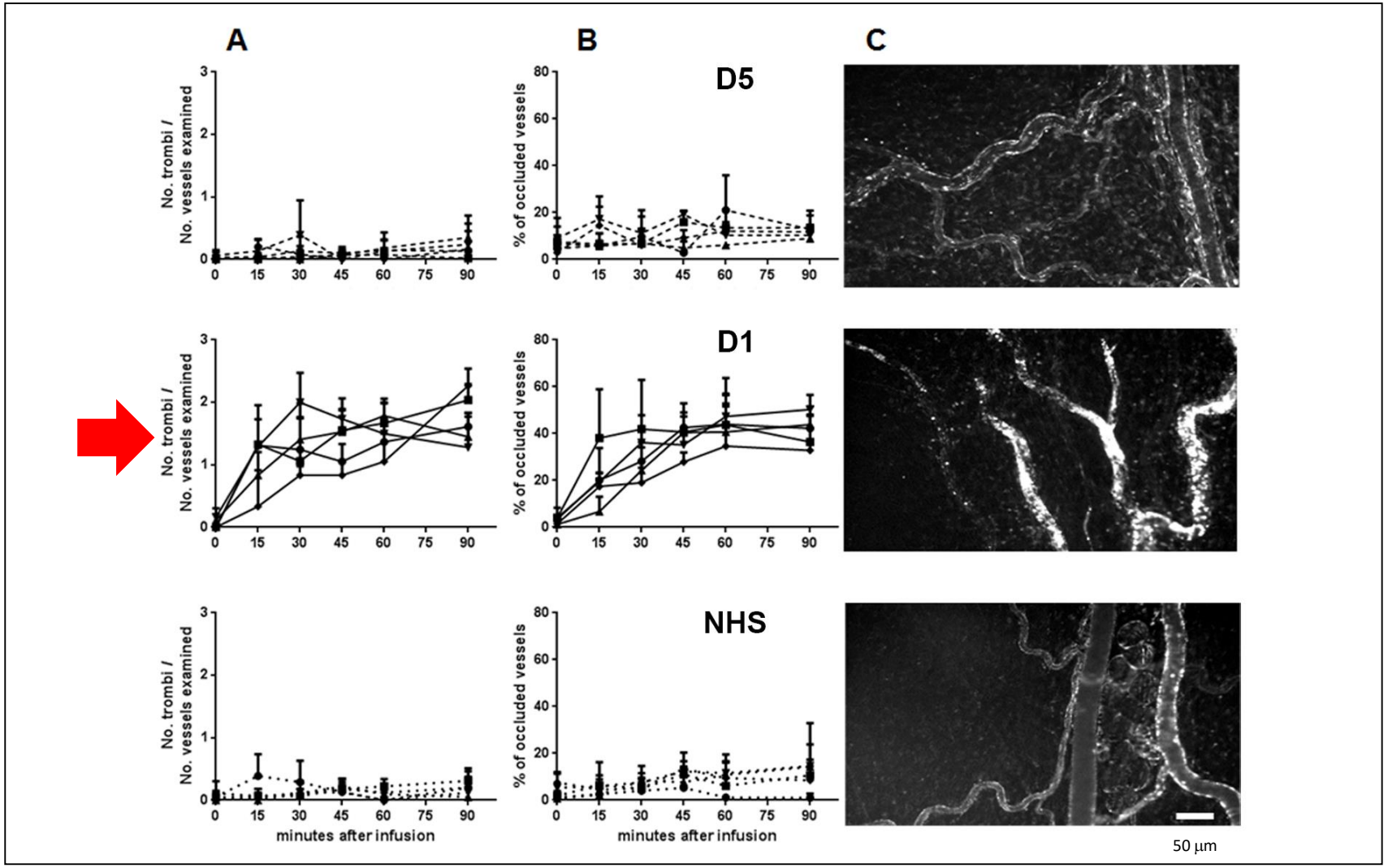
In vivo_1

Do aD5 serum IgG have a thrombogenic effect?



In vivo_1

Do aD5 serum IgG have a thrombogenic effect?

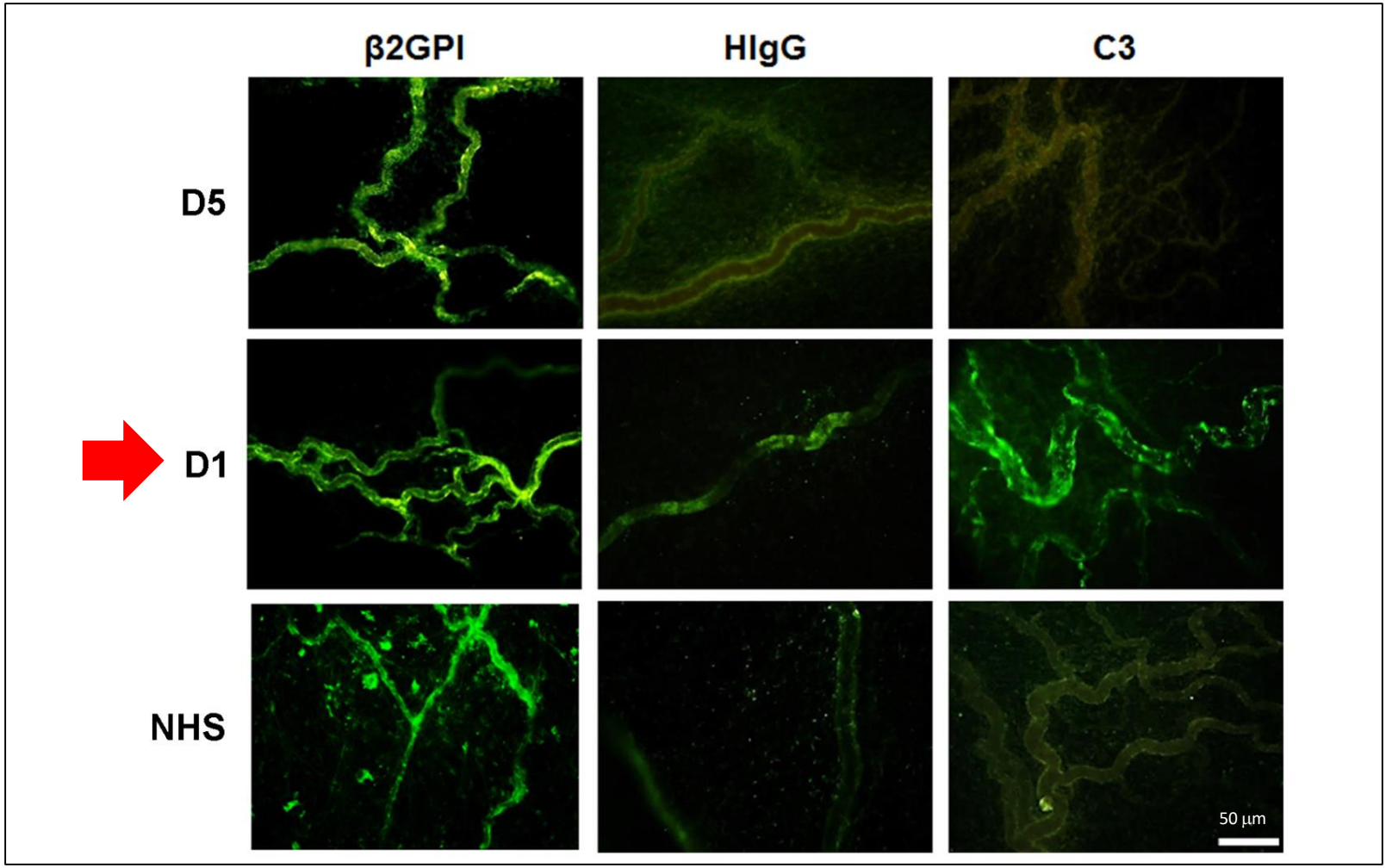


Only aD1 induced blood clots

aD5 failed to induce thrombi in rat

In vivo_1

Do aD5 serum IgG have a thrombogenic effect?



Only rats treated with aD1 clearly showed IgG and C3 deposition on the vessels

In vitro_1

"Do aD5 serum IgG interact with β2GPI bound to the phospholipid?"

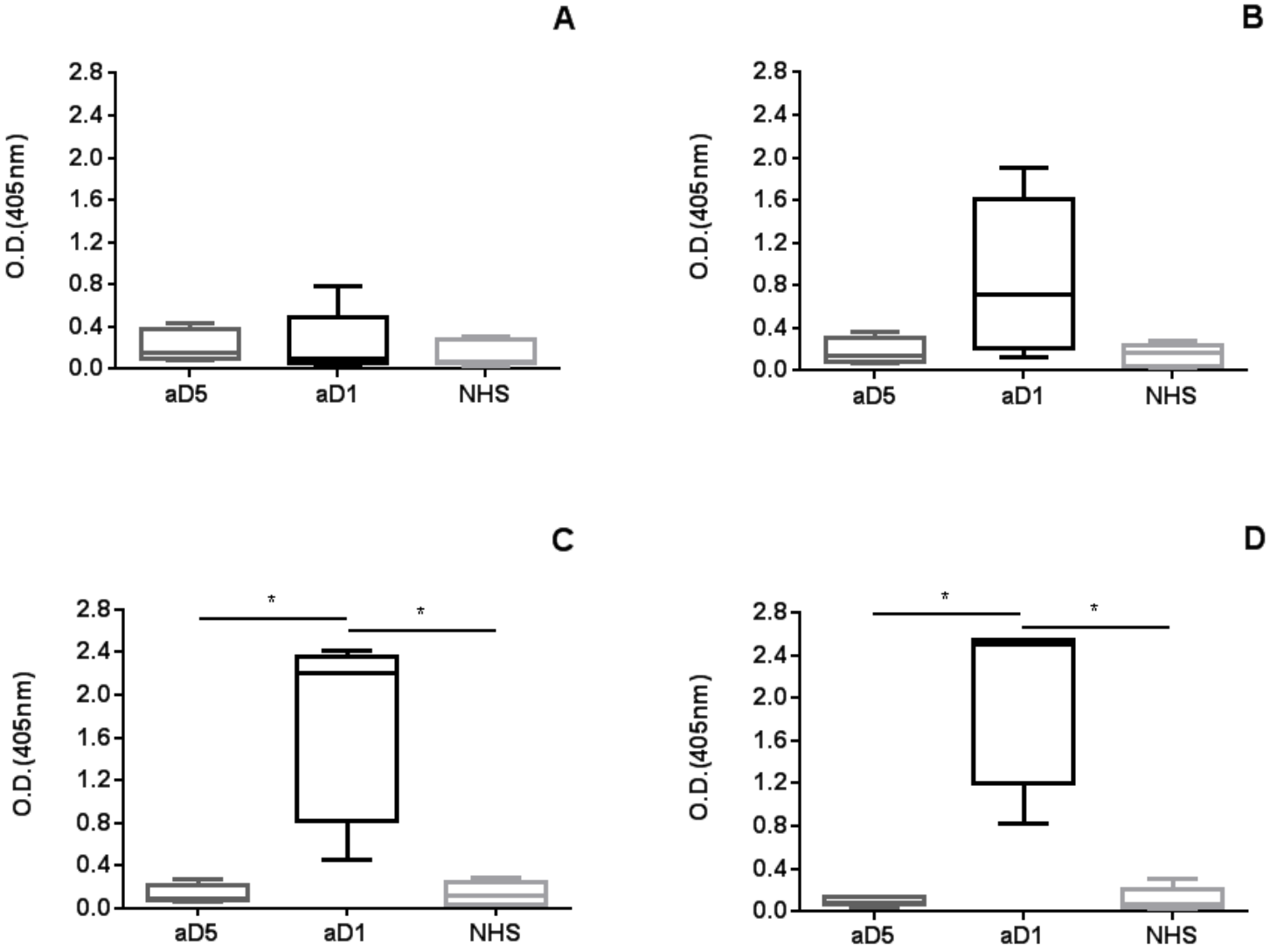
aD5 or aD1 or NHS serum IgG were incubated with Cardiolipin bound to known concentrations of purified human β2GPI

Briefly:

- **coating with Cardiolipin** (+4 °C overnight)
- 2 washes using PBS/BSA 1% and **blocking using PBS/BSA 1% added with known concentrations of β2GPI (RT 2hrs)**
- 2 washes using PBS/BSA 1% (to **remove unbound β2GPI)**
- **Incubation with total IgG (RT 2hrs)**
- 3 washes PBS/BSA 1% (to remove unbound tot IgG)
- Incubation with conjugate (RT 1h 30min)
- 4 washes PBS/BSA 1%
- Incubation with substrate (37°C 30min)
- Reading of the plate (405nm OD)

In vitro_1

"Do aD5 serum IgG interact with β2GPI bound to the phospholipid?"

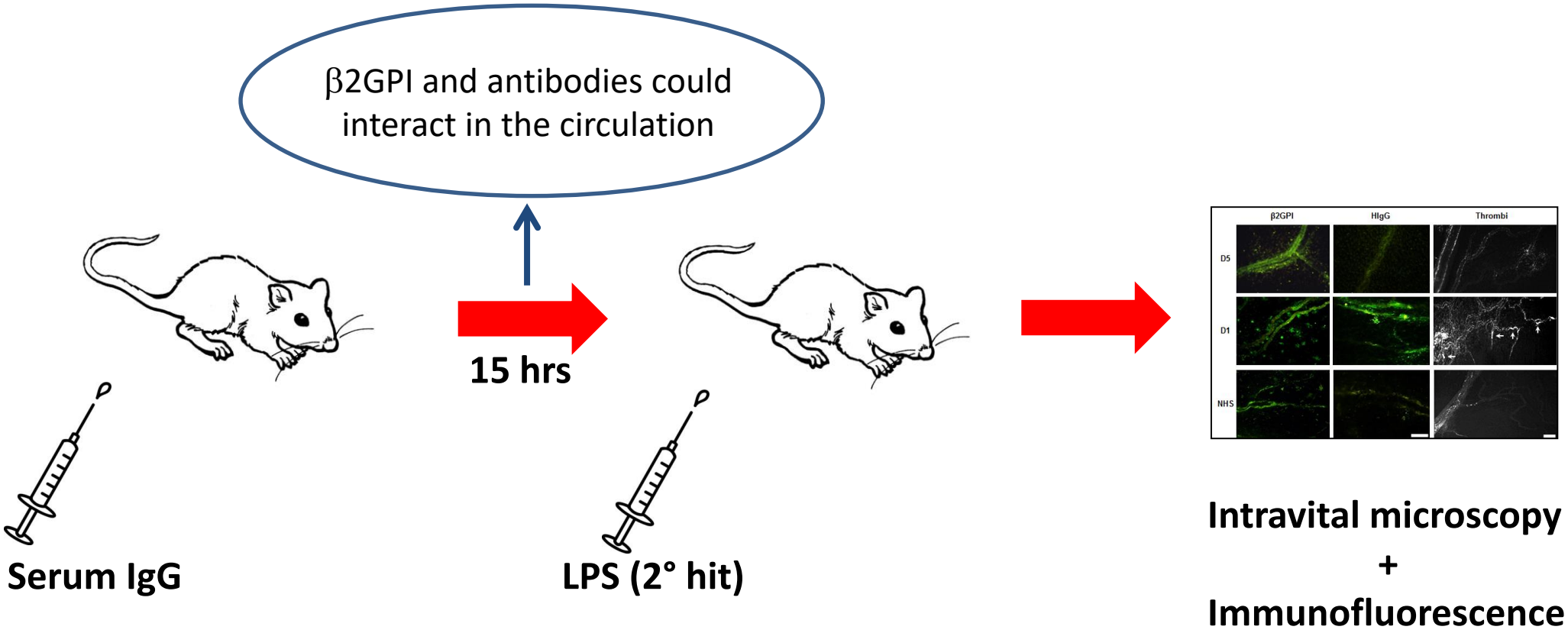


Serum IgG interacting with:
A = no β2GPI bound to Cardiolipin
B = 1 μg/ml β2GPI bound to Cardiolipin
C = 5 μg/ml β2GPI bound to Cardiolipin
D = 75 μg/ml β2GPI bound to Cardiolipin

aD5 fail to interact with β2GPI bound to Cardiolipin

aD1 increase their binding to Cardiolipin raising β2GPI concentrations

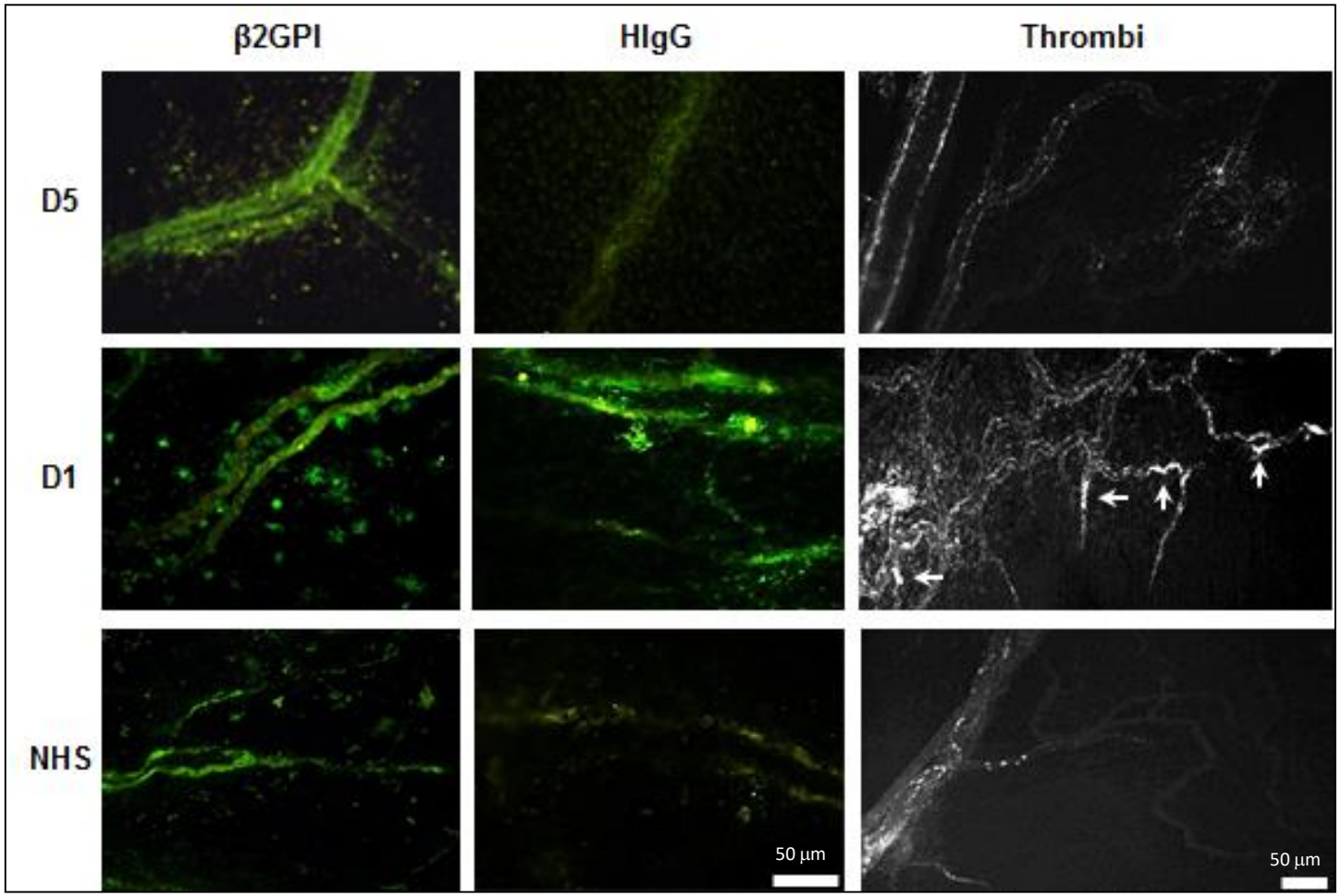
In vivo_2
”Could aD5 modulate β2GPI binding to the endothelium?”



..if the answer was «YES»… we could even dare to consider a potential «protective» role for aD5…

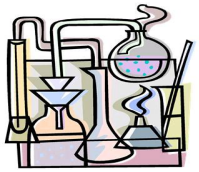
In vivo_2

”Could aD5 modulate β2GPI binding to the endothelium?”



As in the 1° *in vivo* model only aD1 induced blood clots and clearly bound to the vessels

No difference in β2GPI deposition on the vessels of aD5 or aD1 treated rats



In vitro_2
"Do aD5 serum IgG interact with β2GPI in the fluid phase?

aD1 or aD5 or NHS serum IgG were incubated in fluid phase with known concentrations of purified human β2GPI or BSA:

37 °C 1h (orbital shaking) and then 4°C overnight (orbital shaking)

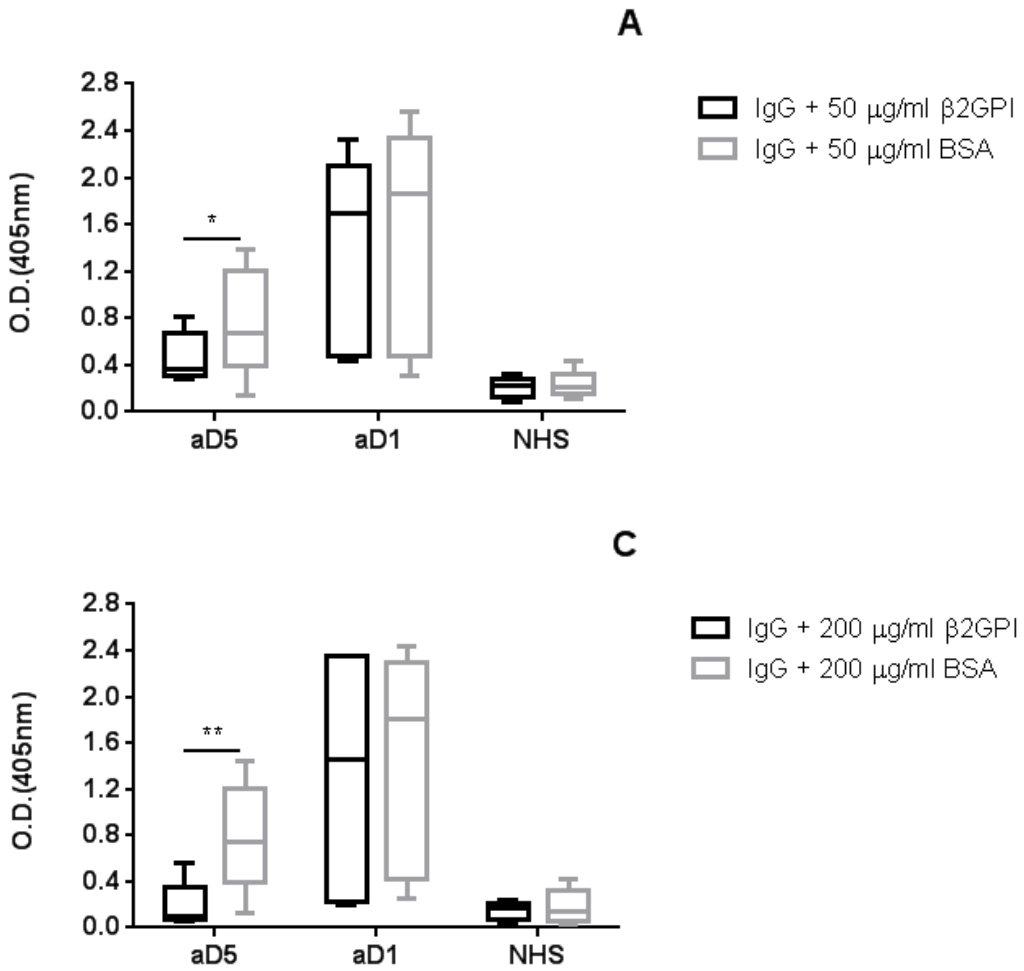
After the overnight **incubation** centrifiuged **samples were tested on a γ irradiated plate directly coated with purified β2GPI**

We evaluated if aD1 or aD5 or NHS serum IgG could interact in distinct way/amount with soluble β2GPI, ending in a different inhibition of their binding to β2GPI whole molecule directly coated on γ irradiated ELISA plate

BSA was the irrelevant control protein

In vitro_2

"Do aD5 serum IgG interact with β2GPI in the fluid phase?



aD5 clearly decrease their reactivity against β2GPI directly coated on the γ-irradiated plate after the o.n. incubation with β2GPI in the fluid phase (comparing BSA as irrilevant protein)

aD1 show a slightly lower reaction against β2GPI directly coated on the γ-irradiated plate, but **not significantly different** if incubated with BSA

in vivo_ex vivo observations:

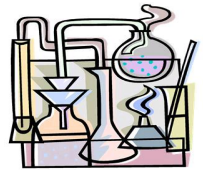
In rats aD5 treated:

- **all the aD5 serum IgG failed to induce thrombi, showing a not pathogenetic role in the thrombotic aPL mediated mechanisms**
- **no complement (C3) deposit and a mild h-IgG binding on the endothelium**

In rats aD1 treated:

- all the aD1 serum IgG confirmed their thrombotic role
- clear complement (C3) deposit and h-IgG binding on the endothelium

- No difference in β2GPI deposit in the 'new' rat model created to observe if the presence of aD1 or aD5 in the circulation could differently modulate the deposition of the target protein on the endothelium



in vitro observations:

- aD5 don’t recognize β2GPI bound to Cardiolipin
- aD5 interact with β2GPI in the fluid phase (circulating β2GPI ?)

… this could be one of the reason why aD5 fail to induce thrombi in vivo...

What we can state…

- Speaking about APS thrombotic events these data support the aD5 not pathogenic role seen in the observational clinical studies and confirm the aD1 pathogenic role

- This experimental confirmation of aD1 and aD5 different roles could reinforce the idea of the aD1/aD4-5 ratio as a useful new biomarker predictive or not of APS clinical manifestations, helping to stratify patients into risk categories

- It’s too early to speak of an «aD5 interfering role» to lower aD1 pathogenic activity…

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…thanks for your attention…