Endothelial procoagulant activity and release of endothelial cell-derived extracellular vesicles driven by antiphospholipid antibodies

Daniel Álvarez[1,2]; Hephzibah E. Winter[1]; Carlos J. Velásquez Franco[3]; Udo R. Markert[1]; Ángela P. Cadavid J.[2]; Diana M. Morales-Prieto[1].

1 Placenta Lab, Department of Obstetrics, Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany.

2 Grupo Reproducción, Departamento Microbiología y Parasitología, Facultad de Medicina, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia.

3 Departamento de reumatología, Clínica Universitaria Bolivariana, Cra 72A #78b -50, Medellín, Colombia.

Objective: Antiphospholipid syndrome (APS) is an autoimmune disease characterized by vascular thrombosis and pregnancy-related morbidity. APS is caused by antiphospholipid antibodies (aPL), but exact underlying pathogenic mechanisms remain unclear. This study explores the aPL-induced activation of endothelial cells, the subsequent release of extracellular vesicles (EVs), and the development of procoagulant effects.

Methods: IgG fractions were purified from serum of 30 patients with different clinical manifestations of APS and 30 control volunteers. Human umbilical vein endothelial cells (HUVECs) were stimulated with IgG (250 µg/mL) in presence of MAPK pathway inhibitors. A plasma recalcification assay was used to determine their coagulation activity. EVs released by HUVECs were isolated by ultracentrifugation and analyzed using flow cytometry and nanotracking analysis (NTA). THP-1 cells (human monocyte cell line) were stimulated with the harvested EVs (1:50 cells:EVs ratio) and their procoagulant activity was assessed by a plasma recalcification assay.

Results: Patients with pure obstetric APS (OAPS) exhibited decreased titers of aCL antibodies (mean: 10.15 IgG phospholipid units [GPL]) and increased responsiveness to treatment with low molecular weight heparin (LMWH) and aspirin (71%) compared to those having vascular and obstetric APS manifestations (VOAPS) (mean: 65.03 GPL, and 29%, respectively). Antibodies from patients with VOAPS and refractoriness to treatment increased the endothelial coagulation potential in a MEK1/2 and p38MAPK pathway-dependent manner (0.03 ± 0.013 vs 0.1 ± 0.03). This activation is accompanied by the release of endothelial-derived EVs which, according to our preliminary results, could increase the procoagulant activity of monocytes.

Conclusions: Patients with pure OAPS are characterized by low aPL titers and high responsiveness to standard treatment. aPL from patients with obstetric manifestations accompanied by vascular thrombosis and refractoriness to treatment activates endothelial cells increasing their coagulation potential. EVs released in this context could increase the procoagulant activity of monocytes amplifying the hypercoagulable state observed in APS.

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3. Departamento de reumatología, Clínica Universitaria Bolivariana, Colombia.

Correspondence:
Daniel.AlvarezJaramillo@med.uni-jena.de

Key acronyms: IgG, immunoglobulin G; NHS, IgG from normal human serum; PM/VT, IgG from patients with vascular and obstetric APS; NR, PM/VT patients non refractory to treatment; R, PM/VT patients with refractoriness to treatment; SB, SB203580 p38MAPK inhibitor; U0, U0126 MEK1/2 inhibitor; Veh, vehicle (DMSO); lEVs, large extracellular vesicles; sEVs, small extracellular vesicles; FC, flow cytometry; RPA, recalcified plasma based assay; IF, immunofluorescence; TEM, transmission electron microscopy.

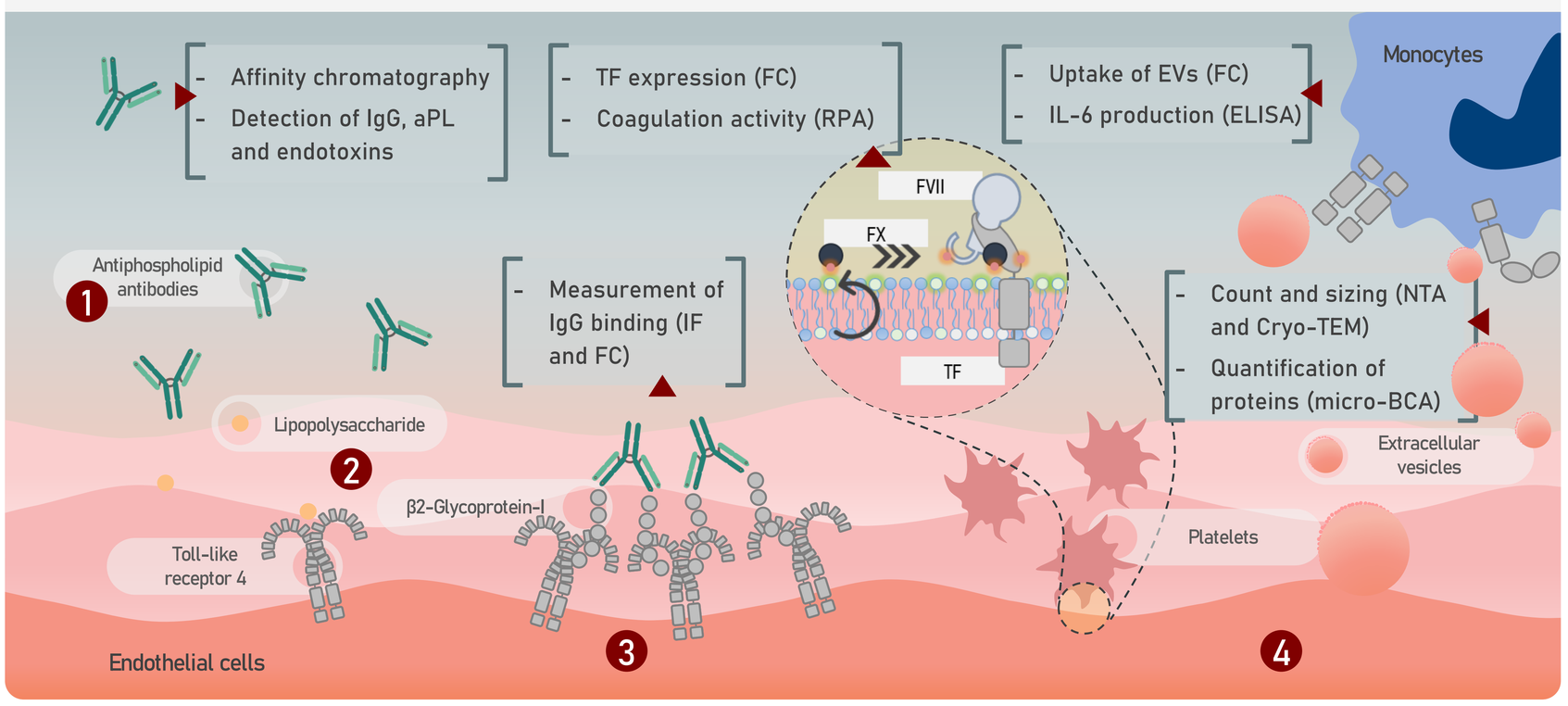
The issue we are focusing on

Introduction

1. Antiphospholipid syndrome (APS) is an autoimmune disease driven by a heterogeneous group of autoantibodies (aPL), which are responsible for vascular (VT) and/or obstetric (PM) manifestations.
2. According to the two-hit hypothesis, aPL requires an additional triggering factor (e.g. LPS) for thrombosis to take place.
3. This second hit should be responsible for increasing TLR4 expression on the endothelial surface, leading to the binding of βGPI-IgG complexes, then enhancing the procoagulant potential.
4. Endothelial activation is accompanied by the release of extracellular vesicles (EVs).

This study investigates the aPL-driven endothelial activation, the consequent release of EVs, and the development of procoagulant effects.

How did we address the question? / Graphical hypothesis and methods



Which are our main findings so far? / Results

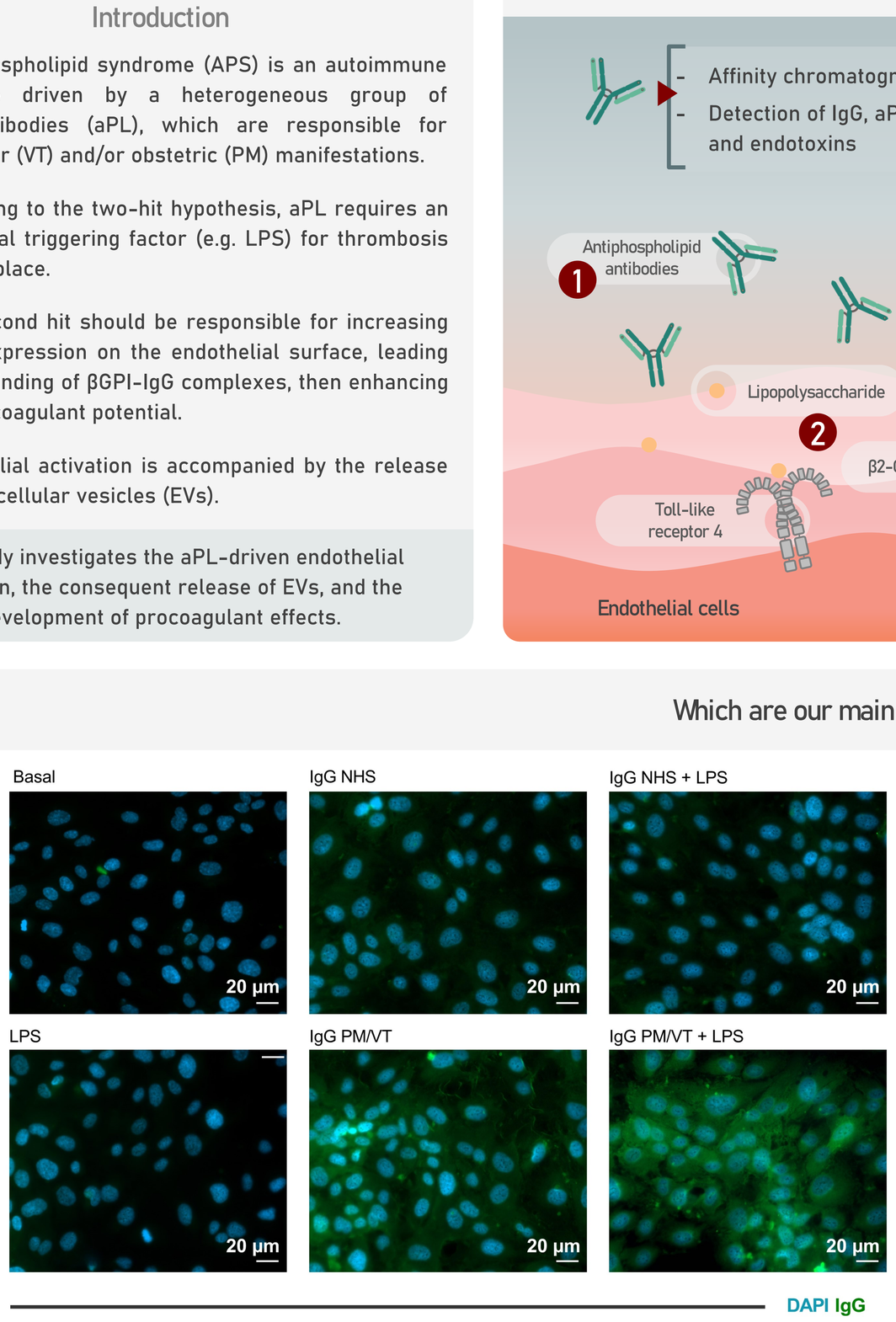


Figure 1. IgG from patients with vascular (VT) and obstetric APS (PM) bind to the surface of endothelial cells even in the absence of a prior stimulus. HUVECs were stimulated with polyclonal IgG ± LPS, and then stained with DAPI (blue) and FITC-conjugated anti-human IgG (green).

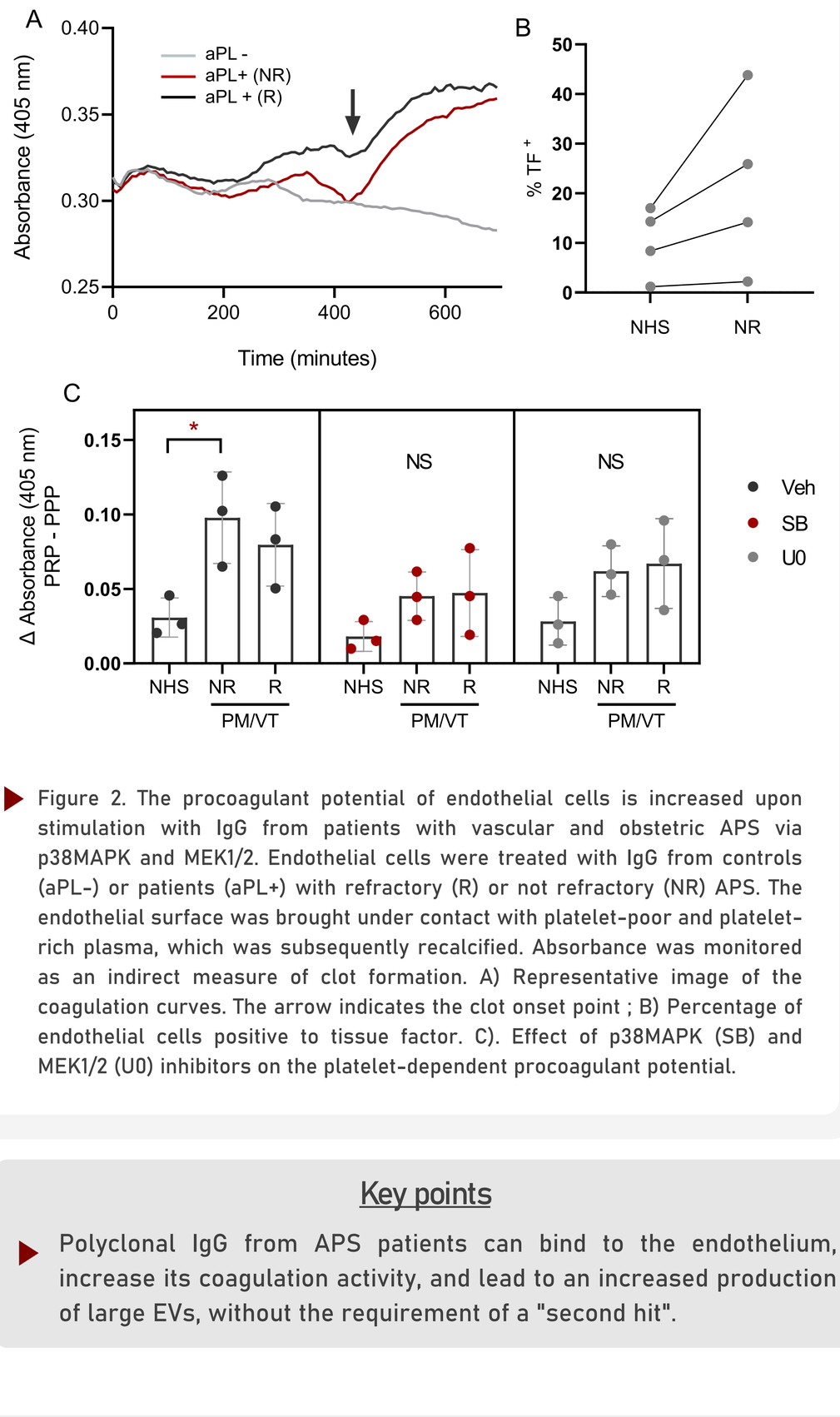


Figure 2. The procoagulant potential of endothelial cells is increased upon stimulation with IgG from patients with vascular and obstetric APS via p38MAPK and MEK1/2. Endothelial cells were treated with IgG from controls (aPL-) or patients (aPL+) with refractory (R) or not refractory (NR) APS. The endothelial surface was brought under contact with platelet-poor and platelet-rich plasma, which was subsequently recalcified. Absorbance was monitored as an indirect measure of clot formation. A) Representative image of the coagulation curves. The arrow indicates the clot onset point ; B) Percentage of endothelial cells positive to tissue factor. C). Effect of p38MAPK (SB) and MEK1/2 (U0) inhibitors on the platelet-dependent procoagulant potential.

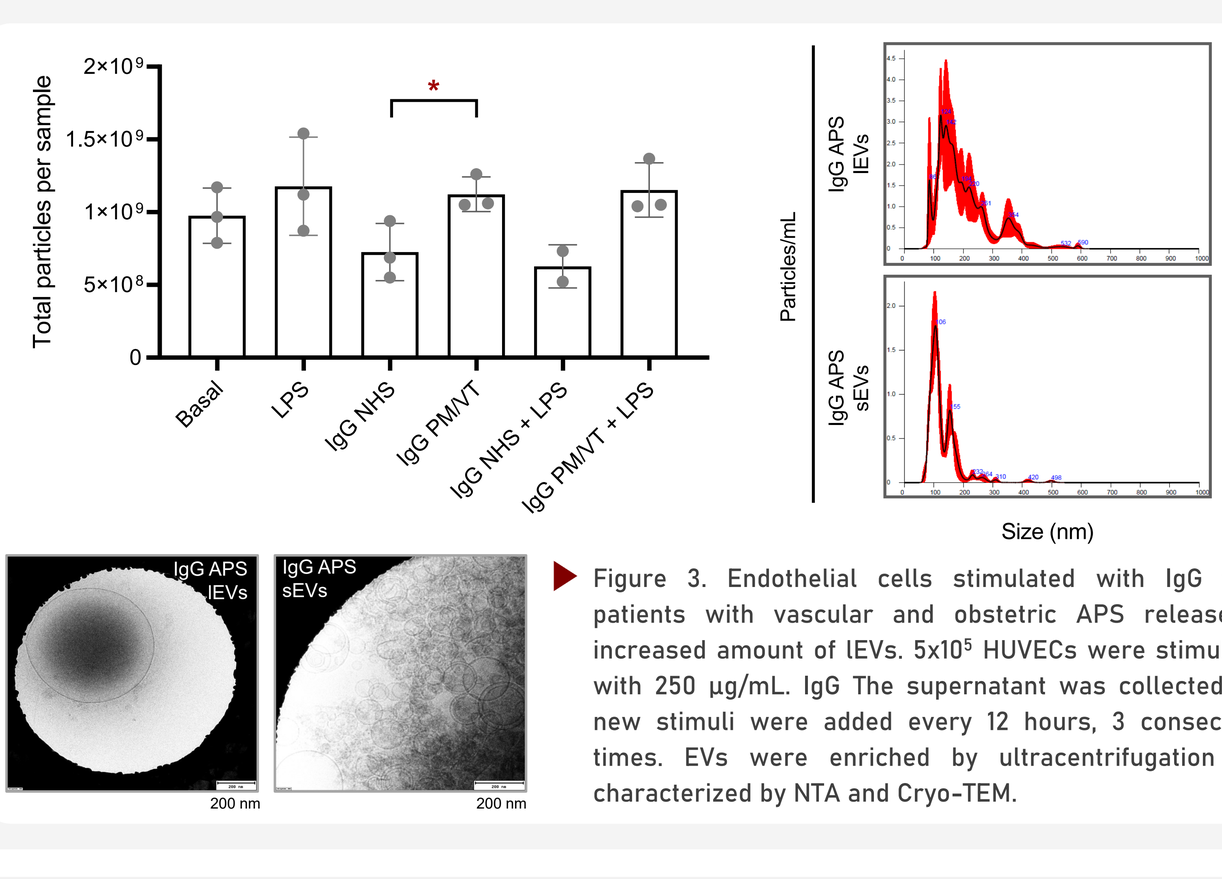


Figure 3. Endothelial cells stimulated with IgG from patients with vascular and obstetric APS release an increased amount of lEVs. $5x10^5$ HUVECs were stimulated with 250 µg/mL. IgG The supernatant was collected and new stimuli were added every 12 hours, 3 consecutive times. EVs were enriched by ultracentrifugation and characterized by NTA and Cryo-TEM.

Key points

- Polyclonal IgG from APS patients can bind to the endothelium, increase its coagulation activity, and lead to an increased production of large EVs, without the requirement of a "second hit".

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