UNIVERSITÀ DEGLI STUDI DI MILANO

Azienda Ospedaliera SAN PAOLO POLO UNIVERSITARIO

ISTITUTO AUXOLOGICO ITALIANO
Istituto di ricovero e cura a carattere scientifico

Complement activation in APS: evaluation of platelet-bound C4d in *ex-vivo* and *in-vitro* studies

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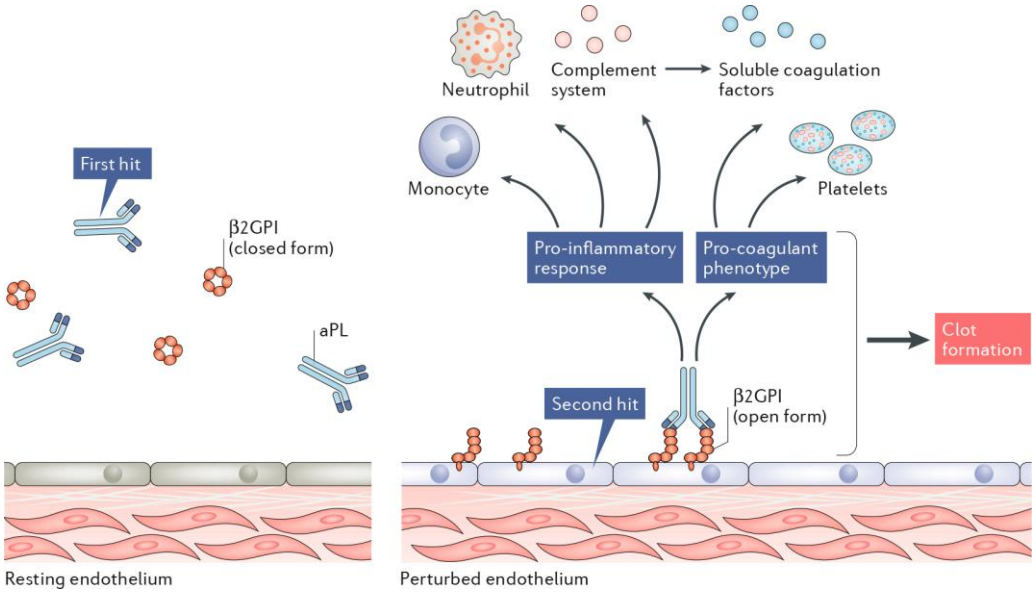
11th Meeting of the European Forum on Antiphospholipid Antibodies
Maastricht, The Netherlands

APSFORUMMAASTRICHT

Complement and APS

Experimental and clinical data support the conclusion that the Complement system is a key factor in the pathogenesis of APS



(Meroni, PL et al. Nat. Rev. Rheumatol., 2018)

Complement and vascular models of APS

Blood. **2005** Oct 1;106(7):2340-6. Epub **2005** Jun 14.

Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor.

Fischetti F[1], **Durigutto** P, Pellis V, Debeus A, Macor P, Bulla R, Bossi F, Ziller F, Sblattero D, Meroni P, Tedesco F.

aPL from patients with APS are able to trigger clotting in the presence of a priming pro-inflammatory factor.

Arthritis Rheum. 2005 Jul;52(7):2120-4.

Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia.

Pierangeli SS[1], **Girardi** G, Vega-Ostertag M, Liu X, Espinola RG, Salmon J.

Lupus. 2012 Dec;21(14):1497-505. doi: 10.1177/0961203312458839.

C6 knock-out mice are protected from thrombophilia mediated by antiphospholipid antibodies.

Carrera-Marín A[1], Romay-Penabad Z, Papalardo E, Reyes-Maldonado E, García-Latorre E, Vargas G, Shilagard T, **Pierangeli** S.

C3 or C5 or C6 k/o animals are protected from aPL induced thrombi

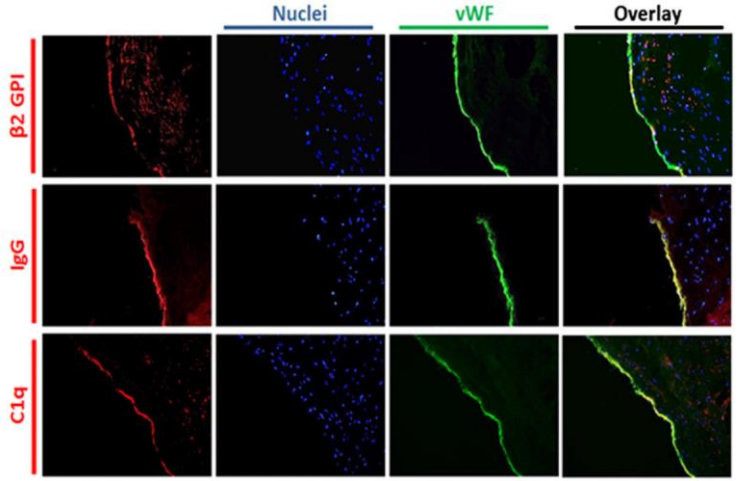
Complement and vascular APS

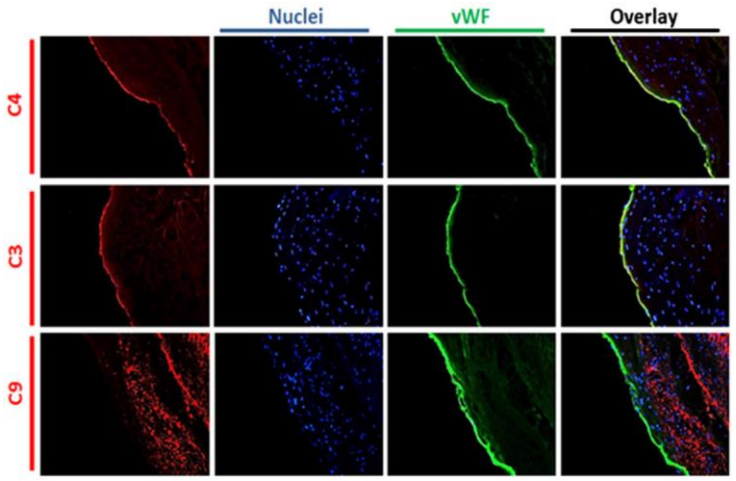
Blood. 2016 Jan 21;127(3):365-7. doi: 10.1182/blood-2015-09-672139. Epub 2015 Dec 7.

Complement activation in antiphospholipid syndrome and its inhibition to prevent rethrombosis after arterial surgery.

Meroni PL[1], **Macor** P[2], Durigutto P[2], De Maso L[2], Gerosa M[3], Ferraresso M[4], Borghi MO[1], Mollnes TE[5], Tedesco F[6].

PAPS patient with arterial thrombosis who underwent arterial surgical bypass.





B2GPI and IgG co-localize in the artery wall

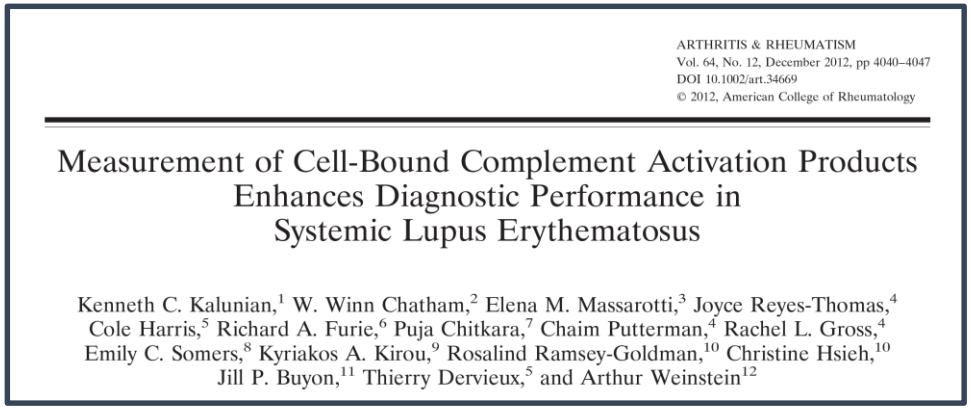
- ✗ Formation of IC
- ✗ Local deposition of C1q, C4 and C3

Indirect demonstration that IC are able to activate the classical complement pathway in-vivo in humans

Aim of the study

- ✗ Complement is involved in APS pathogenesis
- ✗ C3 and C4 serum levels are generally not reduced in APS patients
- ✗ Soluble split complement products are difficult to detect

⇨ Search for split product deposited on cell membranes



ARTHRITIS & RHEUMATISM
Vol. 64, No. 12, December 2012, pp 4040–4047
DOI 10.1002/art.34669

Measurement of Cell-Bound Complement Activation Products Enhances Diagnostic Performance in Systemic Lupus Erythematosus

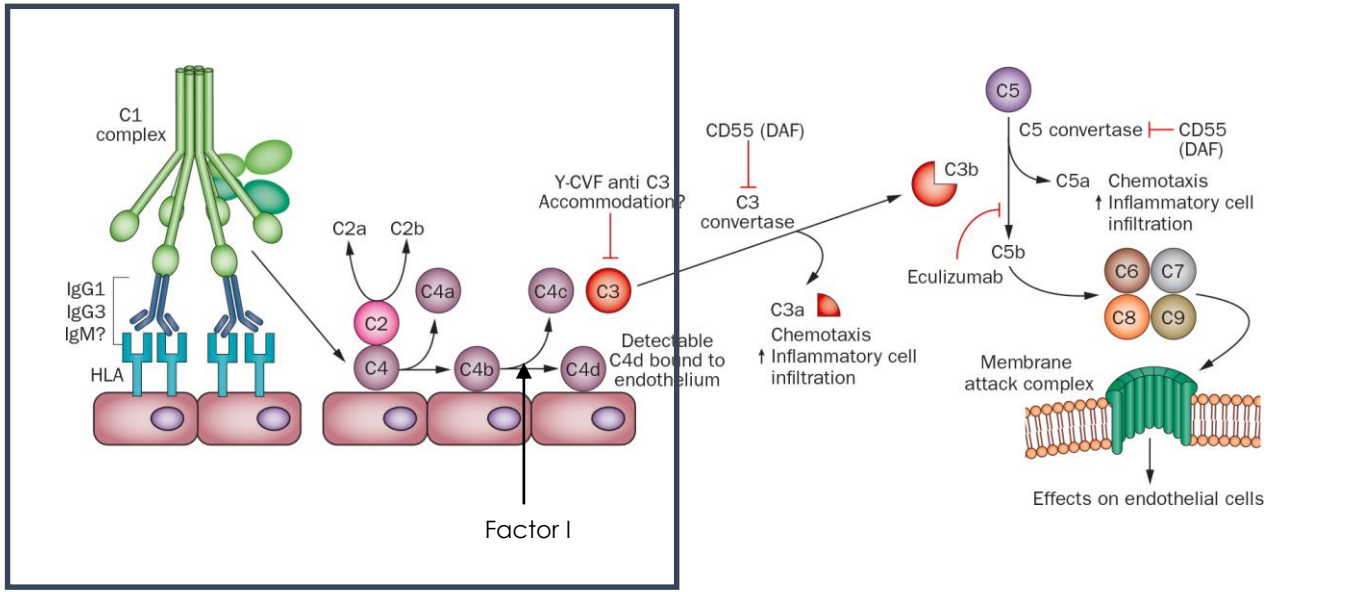
Kenneth C. Kalunian,[1] W. Winn Chatham,[2] Elena M. Massarotti,[3] Joyce Reyes-Thomas,[4] Cole Harris,[5] Richard A. Furie,[6] Puja Chitkara,[7] Chaim Putterman,[4] Rachel L. Gross,[4] Emily C. Somers,[8] Kyriakos A. Kirou,[9] Rosalind Ramsey-Goldman,[10] Christine Hsieh,[10] Jill P. Buyon,[11] Thierry Dervieux,[5] and Arthur Weinstein[12]

⇨ aPL negative SLE patients have higher C4d levels deposited on B cells, erythrocytes and platelets than healthy donors or patients affected of different rheumatic diseases.

⇩

Investigate C4d bound to B cells, erythrocytes and platelets in primary APS patients

Complement cascade



(Stegall MD et al. Nat Rev Nephrol. 2012)

C4d → No biological functions;

→ Binds on cells or tissue near the activation site;

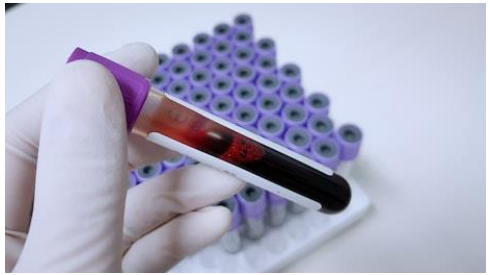
→ No receptors;

→ Covalently bind the membrane surface → the binding does not break spontaneously

Patients

Study population (n= 77)	Primary APS (n= 24)	aPL+ carriers (n= 8)	SAPS (n= 11)	aPL+ SLE (n=18)	aPL- SLE (n= 15)	ITP (n= 11)	aPL neg thrombosis (n= 8)
M/F (%)	11(46)/13(54)	1(12,5)/7(87,5)	0/11(/100)	1(6)/17(94)	3(20)/12(80)	6(54)/5(46)	2(25)/6(75)
Age mean ± SD	48 ± 12	47 ± 11	45 ± 14	42 ± 14	41 ± 15	62 ± 19	78 ± 20
Thrombotic manifestations (%)	19 (79)	0	8 (73)	0	1 (7)	1 (9)	8 (100)
Obstetric + thrombotic APS (%)	1 (4)	-	1 (9)	-	-	-	-
Obstetric APS (%)	4 (17)	-	2 (18)	-	-	-	-
SLEDAI median (min-max)	-	-	4 (0-14)	4 (0-12)	5 (0-16)	-	-
Serum C3 (mg/ml) mean ±SD	88 ±21	95 ± 34	72,5 ± 23	78 ± 23	91,7 ±25	123,5± 35	158,5± 32
Serum C4 (mg/ml) mean ±SD	16 ±9	17 ± 7	15 ± 14	11 ±5	18 ±12	25± 10	31,5± 10
medium/high aCL IgG (%)	21 (87.5)	5 (62,5)	6 (54)	7 (39)	0	0	0
medium/high aCL IgM (%)	2 (8)	1 (12,5)	0	2 (11)	0	0	0
medium/high anti-B2GPI IgG (%)	19 (79)	6 (75)	5 (45)	3 (17)	0	0	0
medium/high anti-B2GPI IgM (%)	4 (17)	3 (37,5)	2 (18)	2 (11)	0	0	0
LAC (%)	21 (87.5)	4 (50)	8 (73)	9 (50)	0	0	0

Ex-vivo protocol

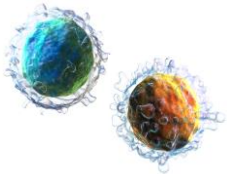


EDTA sample processed within 12 hours from bleeding time

Activated platelets

Platelets

Erythrocytes

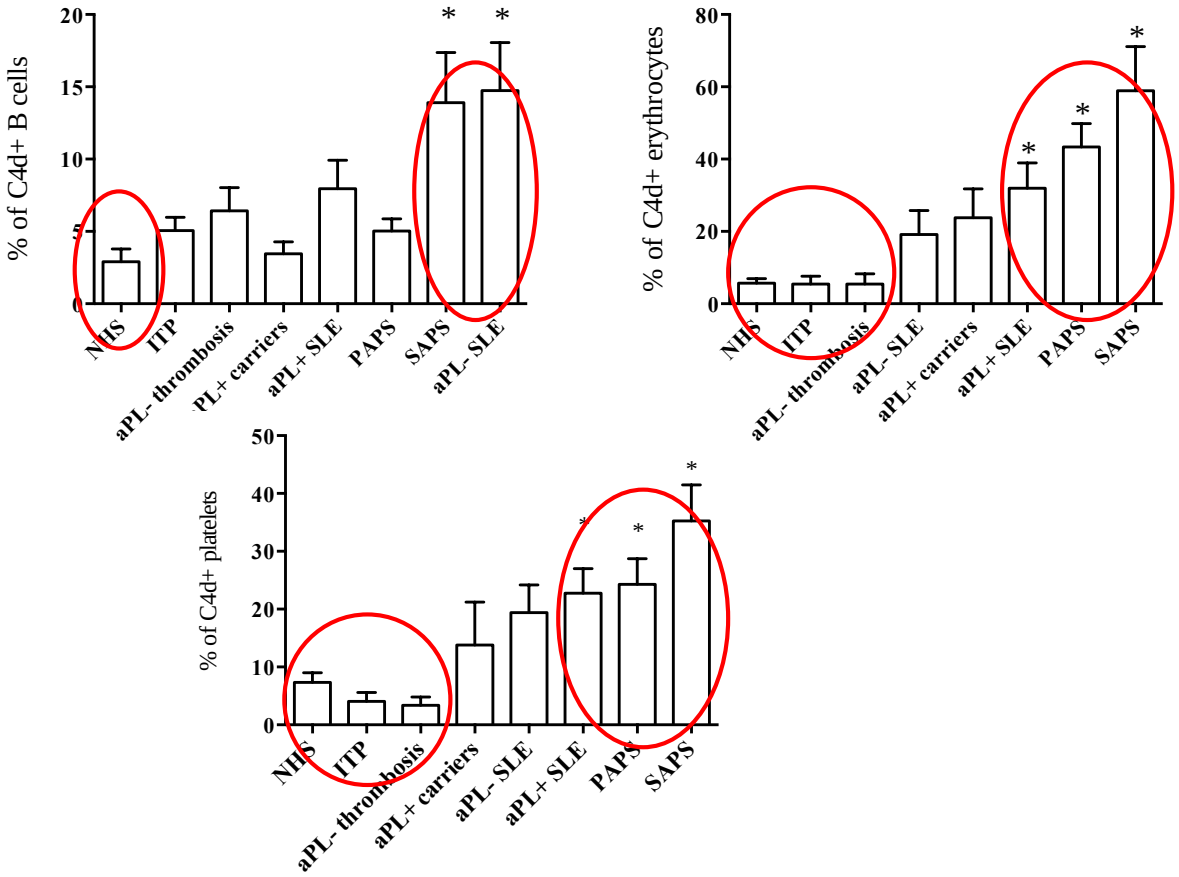


Lymphocytes

Staining:
Platelets: aC4d FITC + CD42b PE
Erythrocytes: aC4d FITC
B cells: aC4d FITC + CD19 PE

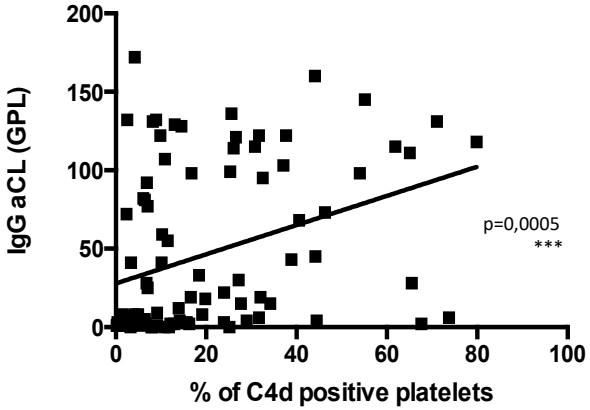
Flow cytometer

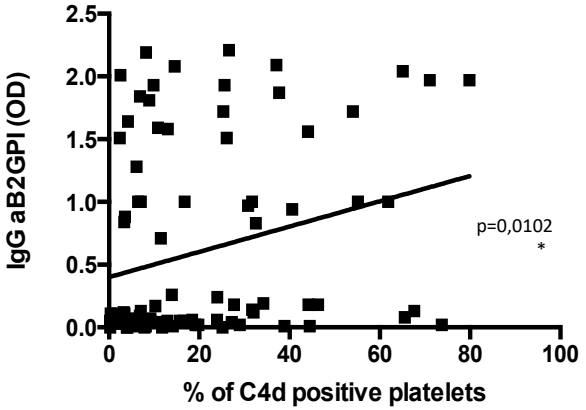
BC4d, EC4d and PC4d

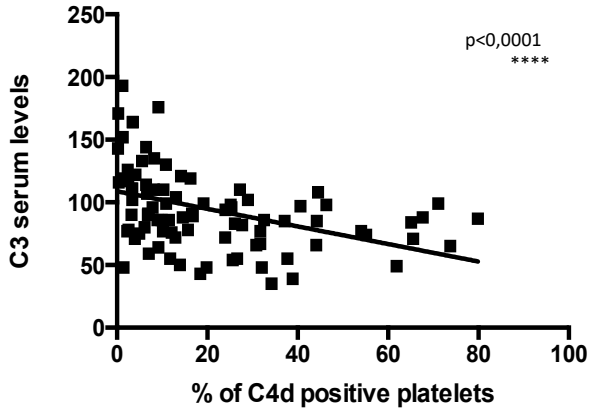


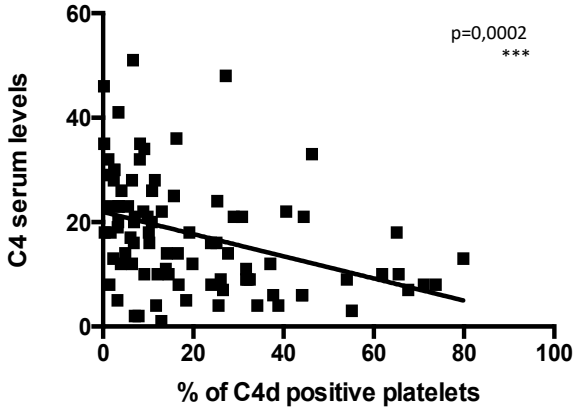
Kruskal-Wallis test + Dunn's test

Platelet correlations

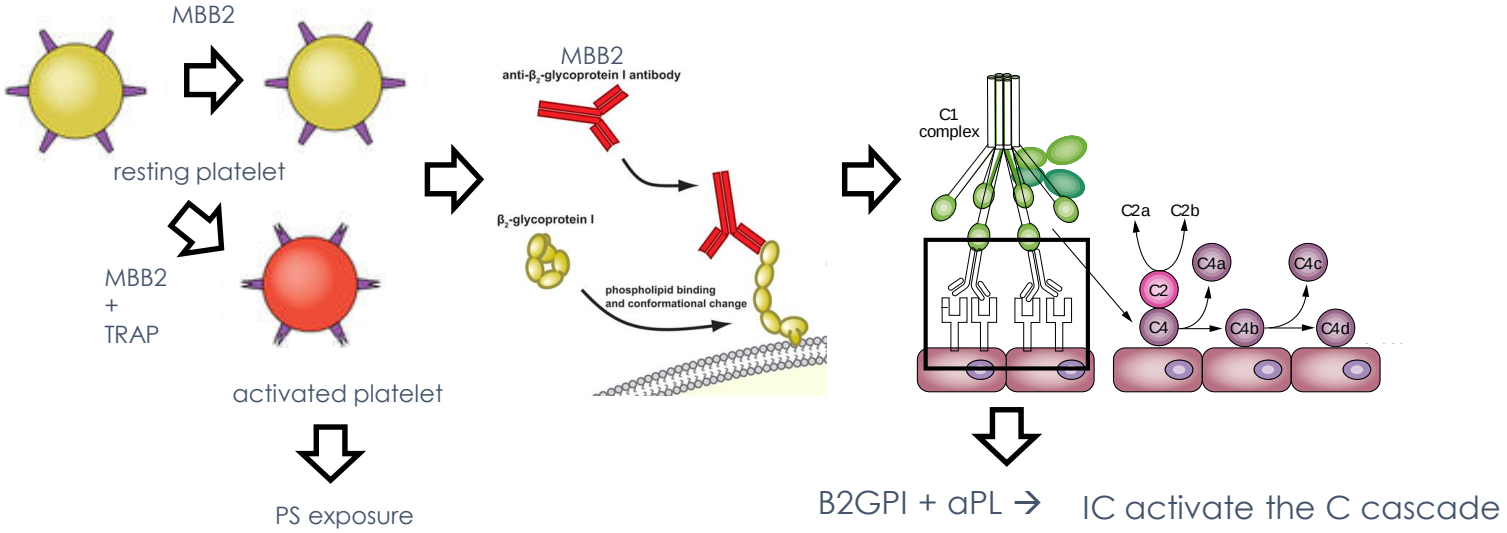








In-vitro model



TRAP: binds to PAR-1 e PAR4 receptors (GPCR)

→ Ca2+ channels
→ membrane flip-flop
→ PS exposition

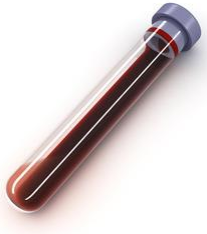
MBB2: a recombinant antibody recognizing the domain I of b2 glycoprotein I induces foetal loss and clot formation in animal models.

THROMBOSIS AND HEMOSTASIS

A non–complement-fixing antibody to β2 glycoprotein I as a novel therapy for antiphospholipid syndrome

Chiara Agostinis,[1] Paolo Durigutto,[2] Daniele Sblattero,[3] Maria O. Borghi,[4,5] Claudia Grossi,[4] Filomena Guida,[2] Roberta Bulla,[2] Paolo Macor,[2] Francesca Pregnolato,[4] Pier Luigi Meroni,[4,5] and Francesco Tedesco[2]

In-vitro protocol



Hirudin blood sample

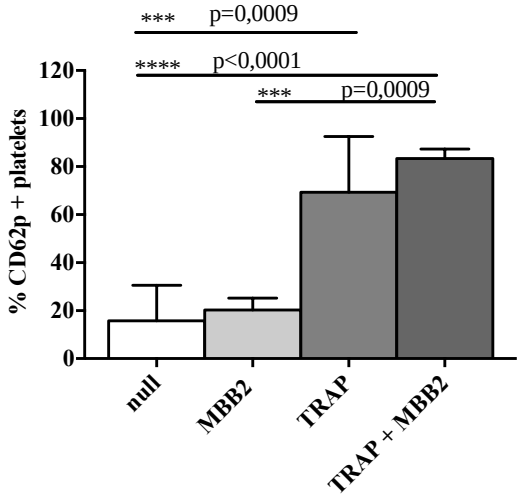
MBB2 ± TRAP stimulation → Incubation 20min at 37°C → Sample dilution

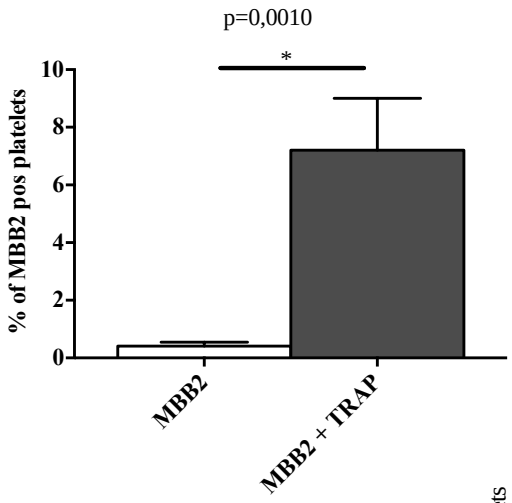
RT Staining:

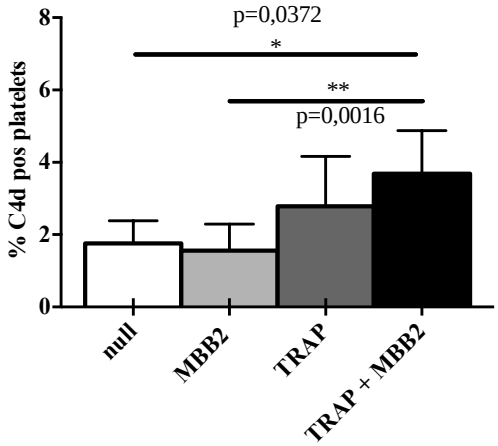
aC4d + anti-mouse APC
aCD42b PE
anti-human FITC
aCD62p Pe-CY7

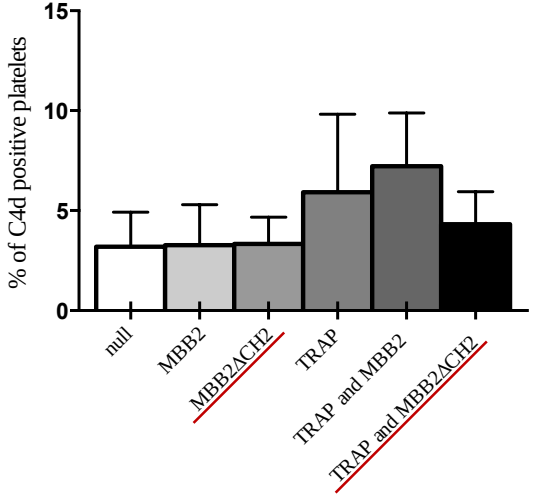
→ Flow cytometer

In-vitro results









Conclusions

Ex vivo

- ✗ aPL are associated with platelet-bound C4d
- ✗ First in-vivo demonstration that the classical complement pathway is activated in PAPS patients

In vitro

- ✗ In presence of a second hit (TRAP) able to activate platelets, and of MBB2, an analogue of aB2, we observe the formation of local Immune Complexes able to activate the complement cascade
- ✗ Complement is not activated when MBB2ΔCH2 is used instead of MBB2

⇩

- ✗ Possible mechanism of C4d deposition on platelets
- ✗ Classical complement activation is involved in APS pathogenesis

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Thank you for your attention