Patient-derived anti-β2GP1 antibodies recognize a peptide motif pattern and not a specific sequence of residues

Philippe de Moerloose,[1] Céline Fickentscher,[1] Françoise Boehlen,[1] Jean-Marie Tiercy,[2] Egbert K.O. Kruithof[1] and Karim J. Brandt[1*]

[1]Division of Angiology and Hemostasis and [2]National Reference Laboratory for Histocompatibility, Transplantation Immunology Unit, Department of Genetic and Laboratory Medicine, University Hospital of Geneva and Faculty of Medicine, Switzerland

*K.J.B is currently affiliated with Division of Cardiology, Department of Internal Medicine, Faculty of Medicine, University of Geneva, Switzerland

 doi:10.3324/haematol.2017.170381

Received: April 10, 2017.

Accepted: May 25, 2017.

Pre-published: May 26, 2017.

Correspondence: karim.brandt@hcuge.ch

Table S1

Accession Number	Protein Name	Gene Name
O60603	Toll-like receptor 2	TLR2
O75581	Low-density lipoprotein receptor-related protein 6	LRP6
O94813	Slit homolog 2 protein	SLIT2
P02749	Beta-2-glycoprotein 1	APOH
P04275	von Willebrand factor	VWF
P10643	Complement component C7	C7
P98155	Very low-density lipoprotein receptor	VLDLR
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	HSPG2
P98164	Low-density lipoprotein receptor-related protein 2	LRP2
Q07954	Prolow-density lipoprotein receptor-related protein 1	LRP1
Q13705	Activin receptor type-2B	ACVR2B
Q14114	Low-density lipoprotein receptor-related protein 8	LRP8
Q9BXB4	Oxysterol-binding protein-related protein 11	OSBPL11
Q9BZF1	Oxysterol-binding protein-related protein 8	OSBPL8
Q9H244	P2Y purinoceptor 12	P2RY12
Q9NPF0	CD320 antigen	CD320

Supplemental methods

Cell culture

Monocytes were isolated from blood buffy coats of healthy volunteers as previously described.[1,2] Monocyte purity routinely consisted of >90% $CD14^+$ cells, <1% $CD3^+$ cells, and <1% $CD19^+$ cells as assessed by flow cytometry. Cells were cultured in RPMI containing 10% Fetal Bovine Serum (FBS; Gibco BRL-Life Technologies). Each experiment was performed with at least three different preparations of monocytes.

HLA Typing

Patients were matched at the second field level typing (high-resolution typing, previously referred to as 4-digit typing) for the HLA-DRB1/B3, DQB1 and DPB1 loci by standard methods: PCR-SSO on microbeads arrays (Luminex Technology, LabType HD, OneLambda, Ingen, Chilly-Mazarin, France), PCR-SSP (Genovision, Milan Analytika AG, Rheinfelden, Switzerland) and SBT (Protrans, Endotell AG, Allschwil, Switzerland).

IgG purification

IgG fractions were isolated from patients plasma with Protein-G CL-4B Sepharose (GE Healthcare) as previously described.[3] To assay for endotoxin and lipopeptide contamination of the IgG fractions, we depleted IgG from the aPLA and IgG-ctl (control) fractions by one step of affinity adsorption to Protein G-Sepharose and tested the remaining supernatant for its capacity for monocyte activation, as previously described [4]. In addition, endotoxin levels were measured by the Limulus Amebocyte Lysate Endochrome Assay (Charles River Laboratories), and were found to be below the detection limit (0.25 EU/mL) for all IgG fractions at the concentration used in the assays. Each experiment was performed with at least 2 different preparations of IgG.

Tetramer staining of class II-peptide

Staining was performed following the Benaroya research institute's protocol (https://tetramer.benaroyaresearch.org/more-inforesources/protocols). Briefly, we performed antigen specific amplification with domains I-II, *i.e* cells were cultured in the presence of antigens (10μg/ml) for 10 days (pulse), prior to tetramer staining as described below. 10 μg/mL of PE-labeled Class II tetramer were added to the cells for 3 hours at 37 °C in the dark. Subsequently, cells were stained with fluorochrome (AF647)-labeled anti-CD4 for 30 minutes on ice before flow cytometry analysis.

TNF production

The cells were treated with recombinant domains or peptides of β_2GP1 at 10 μg/ml prior to incubation with aPLA or control IgG. Supernatants were collected for TNF quantification by ELISA (R&D System).

Western blot

Total cell lysates were prepared and subjected to Western blot analysis as described previously.[3] The blots were probed with anti-cMyc (Zymed). Secondary antibodies conjugated to IR800CW (Rockland) were used. Antibody-bound proteins were detected and quantified by the Odyssey system (Li-Cor).

1. Brandt KJ, Fickentscher C, Kruithof EK, de Moerloose P. TLR2 ligands induce NF-kappaB activation from endosomal compartments of human monocytes. PLoS One. 2013;8(12):e80743.
2. Mentzer SJ, Guyre PM, Burakoff SJ, Faller DV. Spontaneous Aggregation as a Mechanism for Human Monocyte Purification. Cellular Immunology. 1986 Sep;101(2):312-9.
3. Brandt KJ, Fickentscher C, Boehlen F, Kruithof EK, de Moerloose P. NF-kappaB is activated from endosomal compartments in antiphospholipid antibodies-treated human monocytes. J Thromb Haemost. 2014 May;12(5):779-91.
4. Satta N, Kruithof EK, Fickentscher C, et al. Toll-like receptor 2 mediates the activation of human monocytes and endothelial cells by antiphospholipid antibodies. Blood. 2011 May 19;117(20):5523-31.