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

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| 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. 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. 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. 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 β2GP1 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).

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