

UNITE DE RECHERCHE 698
HEMATOLOGIE, BIO INGENIERIE,
REMODELAGES
CARDIOVASCULAIRES
HOPITAL BICHAT



Suspecting a GPVI deficiency

How to proceed

Martine Jandrot-Perrus, Bénédicte Dumont, Stéphane Loyau, Nadine Ajzenberg

This protocol has been established at U698 Inserm and the Hematology laboratory (AP-HP) Bichat Hospital Paris.

Reagents for platelet aggregation (convulxin) and flow cytometry (anti-GPVI antibodies) as well as protocols, can be provided by U698 in the frame of a collaborative study on GPVI abnormalities.

Biochemical (i.e. immunoblot, antibody purification and characterization...) and genetic analysis could be performed on samples sent to U698). Eventually, the help of an expert from the laboratory can be proposed for experiments on fresh blood.

In a patient presenting an isolated defect in collagen-induced platelet aggregation on PRP

After ruling out the ingestion of drugs interfering with TXA2 synthesis and a deficiency in dense granule content/secretion,

Initial tests to be performed

- Platelet aggregation on PRP with convulxin. A deficient GPVI response could be confirmed using the activating anti-GPVI mouse IgG 9O12.
- Control the defect on washed platelets with collagen / convulxin
- Flow cytometry : quantification of major platelet glycoproteins with the Platelet GP screen test kit from Biocytex (France). To quantify GPVI, use the 3J24.2 monoclonal antibody at 10 µg/ml in the same conditions as described for the other GP

Whatever the possible diagnostic, keep all PPP samples frozen!

Case 1: Deficient aggregation to collagen and convulxin associated with a decreased GPVI expression

- Confirm the GPVI deficiency by immunoblotting on whole platelet lysates
- Check the expression of the FcR γ chain by immunoblot
- Search for anti-GPVI antibodies
 - Analysis a platelet bound human IgG (there is not yet an available MAIPA for GPVI)
 - Mixing experiment in aggregometry: mix control PRP with patient PPP (v/v) or control PPP in the aggregometer. Spontaneous platelet aggregation with the patient's PPP can occur after a more or less long lag time (wait until 30 min) is in favour of an activating antibody. If no spontaneous aggregation occurs, then trigger aggregation by collagen, convulxin and a control agonist (TRAP for example). A reduced response in the presence of patient's PPP is in favour of an inhibitory antibody.
 - An antibody could be characterized in binding experiments to recombinant GPVI and its class determined.
 - An antibody can be then purified from plasma according to standard procedure and tested for its properties on platelet function and on GPVI behaviour.
- Search for a shedding of GPVI:
 - On immunoblots of whole platelet lysates using antibodies specific to the extracellular and cytoplasmic GPVI domains
 - In PPP by measuring soluble GPVI.
 - In vitro using the purified antibodies

More specialized tests :

- Platelet adhesion on collagen or convulxin

* In static conditions using washed platelets and a colorimetric assay.

Contribution of $\alpha 2\beta 1$ integrin to platelet adhesion to collagen can be prevented by working in the absence of calcium and magnesium. Platelet aggregation can be prevented in the presence of an antagonist of the $\alpha \text{IIb}\beta 3$ integrin(RGDS or Abxicimab)

*In flow conditions using whole blood in flow chambers on immobilised collagen.

- Platelet procoagulant activity: we recommend to analyse residual prothrombin (often increased) and if possible to perform thrombin generation tests on PRP.

Genetic analysis:

If there is no evidence in favour of an antibody

- RT-PCR on platelet mRNA to analyse transcription of gp6
- Genomic sequence of gp6 : exons and exons/introns junctions and promoter to search for polymorphisms and mutations. If a non-described mutation is found, its causative effect should be controlled by expressing recombinant mutant GPVI and analysis of its properties.

In case of a confirmed antibody, we also recommend to sequence gp6 in order to establish a register of such patients and to analyse whether there is an association between polymorphisms and development of antibodies.

Case 2: Deficient aggregation in response to collagen and convulxin, NOT associated with a deficiency in GPVI expression

- A decreased adhesion to collagen in static conditions when $\alpha 2\beta 1$ and $\alpha \text{IIb}\beta 3$ are blocked could indicate a loss of function mutation. Gp6 sequencing needed

- Another possibility is a defect in the GPVI-coupled signalling pathway.

Control that the patient is not treated by drugs targeting signalling proteins.

Control the association of GPVI with the FcR γ chain by immunoprecipitation and immunoblotting.

Analyse the Fc γ RIIA pathway (identical to the GPVI pathway) using the monoclonal antibody IV.3 and an anti-mouse IgG or Fab'2 .

More specialized studies on signalling proteins would have to be discussed.

Bibliography:

Examples of mutant GPVI [1, 2]

Examples of anti GPVI antibody [3-6]

Examples of deficient signalling [7, 8]

Example of drug –induced signalling defect [9]

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