





Platelet activation leads to an altered expression of already constitutively expressed surface glycoproteins. Increased numbers of GPIIb-IIIa complexes and reduced numbers of GPIb-IX complexes result from bi-directional trafficking of these glycoproteins between the cell surface, the surface-connected canalicular system and intracellular storage (6). Inside-out signaling leads to conformational changes of GPIIb-IIIa complexes, exposing conformation-dependent activation epitopes with high affinity for their ligands (7). The release reaction of platelets is associated with the neo-expression of  $\alpha$ -granule glycoproteins such as CD62P or CD63. Measuring the expression of these antigens on circulating platelets reflects not only the activation state of the platelets but also to what extent secretion has occurred.

### Platelet Activation Assays

Platelet hyper-reactivity and/or circulating activated platelets have been associated with many cardiovascular, infectious, metabolic and auto-immune disorders in several research studies (8-11). Previous methods used to detect platelet activation (for example, aggregometry, radioimmunoassay,  $\alpha$ -thromboglobulin and platelet factor 4 measurement) suffer from several drawbacks. Conversely, whole blood flow cytometric assay using platelet associated monoclonal antibodies presents several advantages:

- ◆ direct assessment of *in vivo* activation states and *in vitro* platelet reactivity to agonists;
- ◆ minimal manipulation prevents activation artifacts;
- ◆ detection threshold of 1% activated platelets or less, even with small sample volume.

### Analysis of Platelet Membrane Glycoproteins by Flow Cytometry

Flow cytometry is a sensitive and rapid research tool for the study of both inherited and acquired platelet disorders by quantitation of the surface expression of the principal adhesion and aggregation receptors (GPIb-IX, GPIIb-IIIa) and of secreted platelet proteins (CD62P, CD63, thrombospondin, fibrinogen) (12). Conformational changes in platelet glycoproteins, especially GPIIb-IIIa, can be measured using monoclonal antibodies recognizing receptor-induced binding sites (RIBS) on the ligand, or ligand-induced binding sites (LIBS) on the receptor (13). Increased amount of platelet-bound ligands such as fibrinogen, von Willebrand Factor or thrombospondin can be quantified using ligand-directed antibodies.

### Future

Beside the large current applications of platelet membrane glycoproteins in fundamental research, the accurate understanding of their involvement in platelet function disorders (inherited or acquired) may rapidly develop towards new anti-thrombotic strategies. The development of inhibitors for GPIIb-IIIa function is one of the most eagerly anticipated advances for future exploration of platelet activation states (14).

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