

**Monday, July 13, 1981**

## **Oral Presentations**

### **Platelets – II**

#### **Membrane Glycoproteins**

**08:00–09:30 h**

### **Platelets – III**

#### **Receptors**

**09:45–11:00 h**

**Grand Ballroom Centre**

## **0057**

**08:15 h**

THE REDUCED AGGREGATION RESPONSE OF BERNARD-SOULIER PLATELETS TO THROMBIN MAY BE RELATED TO AN ABNORMAL GLYCOPROTEIN V. A.T. Nurden and D. Dupuis. U150 INSERM, Hôpital Lariboisière, Paris, France.

Both platelet membrane GP Ib and GP V have been proposed as receptors for the activation of human platelets by thrombin. Bernard-Soulier (B-S) platelets exhibit a reduced aggregation response to thrombin with a lag phase that precedes aggregation. When B-S platelets, whose surface proteins had been labelled with ( $^{125}$ I), were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by PAS-staining, Coomassie blue staining or autoradiography, the apparent absence of GP Ib and the normal presence of GP IIb, IIIa and IIIb was demonstrated. On the basis of such studies several authors have stated that "GP I" is the thrombin receptor. However, GP V is not located by the above procedure, requiring more sensitive analytical methods for its detection. To meet this requirement washed platelets isolated from 3 B-S patients have been treated sequentially with neuraminidase, galactose oxidase and sodium ( $^3$ H)-borohydride. The labelled platelets were analysed by SDS-PAGE using 7–12% gradient acrylamide gels and the ( $^3$ H)-labelled GP's located by fluorography. In addition to the GP Ib defect the platelets of each B-S patient were lacking the band corresponding to GP V of normal platelets. In agreement with previous studies we observed that when ( $^3$ H)-labelled normal human platelets were incubated with thrombin GP V (Mr=82,000) was hydrolysed, and that this was accompanied by the appearance of a labelled glycopeptide (Mr=69,500) in the supernatant. When ( $^3$ H)-labelled B-S platelets were treated with thrombin no labelled glycopeptide was located. GP V could therefore be either absent from B-S platelets or have a modified carbohydrate composition rendering it insensitive to the analytical procedure used. Interpretations into the reduced aggregation response of B-S platelets to thrombin should be extended to include a possible GP V defect.

## **0056**

**08:00 h**

ADDITIONAL GLYCOPROTEIN DEFECTS IN GLANZMANN'S THROMBASTHENIA PLATELETS. J.L. McGregor, K.J. Clemetson, E. James, A. Capitanio, M. Dechavanne and E.F. Lüscher. INSERM Unité 63, Fac. de Med. Alexis Carrel, Lyon, France; Theodor Kocher Institute, University of Berne, Switzerland and Haemophilia and Thrombosis Centre, Milan, Italy.

Glanzmann's thrombasthenia (G.T.) platelets are deficient in 2 major membrane GP (IIb and IIIa). In order to investigate if these are the only defects in this disorder, platelets from G.T. patients and from healthy donors were isolated, washed and surface-labelled by techniques specific for protein or for sugars (sialic acid or penultimate galactose/N-acetylgalactosamine residues). Labelled or unlabelled platelets were solubilized in sodium dodecyl sulphate (SDS) and separated by 2-dimensional polyacrylamide gel electrophoresis, first according to isoelectric point and then according to molecular weight. Glycoproteins from unlabelled platelets separated by 2-dimensional electrophoresis were identified by binding of  $^{125}$ I-labelled *Lens culinaris* lectin (mannose, glucose specific) GPIIbA1 and IIIaA1 were absent in one G.T. patient while in others lower amounts of 2 GP were found in positions similar to these GP. Major membrane GP (IbA1, IbB1 and IIIbA1) had more intensely labelled terminal sialic acid moieties in G.T. platelets than in normals. A major membrane GP designated Ic had an altered pI and its penultimate galactose/N-acetyl galactosamine residues labelled more intensely in G.T. platelets than in controls. One high M.Wt. GP and a number of lower M.Wt. GP (IVa, IVb and VII) normally found in platelets of healthy donors were absent in G.T. platelets. These results indicate strongly that there is a major perturbation of the platelet surface in G.T.

## **0058**

**08:30 h**

TRYPTIC PEPTIDE MAPS OF THE MAJOR HUMAN BLOOD PLATELET MEMBRANE GLYCOPROTEINS, Ia, IIa, IIb, IIIa, IIIb and IIIc. J.L. McGregor, K.J. Clemetson, E. James, P. Clezardin, M. Dechavanne and E.F. Lüscher. INSERM Unité 63, Fac. de Med. Alexis Carrel, Lyon, France and Theodor Kocher Institute, University of Berne, Switzerland.

Some major platelet membrane glycoproteins separated by 2-dimensional polyacrylamide gel electrophoresis (isoelectric focusing, discontinuous SDS-gel electrophoresis) have been characterized by 2-dimensional tryptic peptide mapping. Human platelets were isolated, washed and surface-labelled by lactoperoxidase-catalyzed iodination. Labelled platelets were solubilized in SDS and separated by 2-dimensional gel electrophoresis under non-reducing conditions in the 1st dimension and either reducing or non-reducing conditions in the 2nd dimension. Alternatively, labelled platelets were solubilized in sodium deoxycholate (1%) and the glycosylated components were isolated by lectin affinity-chromatography on *Lens culinaris* lectin or concanavalin A lectin and separated by 2-dimensional gel electrophoresis. The glycoproteins were cut out from 6% polyacrylamide gels, after being identified with Coomassie blue staining and indirect autoradiography by their pI and molecular weight. Tryptic maps were prepared according to the method of Elder *et al.* The tryptic maps of GPIa, IIa, IIb, IIIa, IIIb and IIIc are different, with each showing a characteristic pattern with the possible exception of Ia and IIb which showed certain similarities in both the reduced and non-reduced states. GPIIa which is clearly separated under non-reducing conditions, appears from its tryptic map to be present in the Ib region when reduced. Thus this technique clearly identifies each GP by a parameter in addition to pI and molecular weight.