Role of GPIbα Clustering and N-linked Carbohydrates in the Clearance of Refrigerated Platelets.

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i Mikrobiologens föreläsningsal, våning 3, Guldhedsgatan 10A, Göteborg, fredagen den 27 oktober 2006, kl 09.00

av

Emma Josefsson

Fakultetsopponent: Professor Bernhard Nieswandt, Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Würzburg, Germany

Avhandlingen baseras på följande arbeten:

I **The Macrophage $\alpha_M\beta_2$ Integrin α_M Lectin Domain Mediates the Phagocytosis of Chilled Platelets**
Emma C. Josefsson, Harry H. Gebhard, Thomas P. Stossel, John H. Hartwig, Karin M. Hoffmeister
J Biol Chem., 2005; 280 (18): 18025-18032

II **Glycosylation Restores Survival of Chilled Blood Platelets**
Karin M. Hoffmeister, Emma C. Josefsson, Natasha A. Isaac, Henrik Clausen, John H. Hartwig, Thomas P. Stossel
Science, 2003; 301: 1531-1534

III **Differential Changes in the Platelet vWf Receptor Following Refrigeration for Short or Long Periods**
Emma C. Josefsson, Viktoria Rumjantseva, Herve Falet, Claes Dahlgren, John H. Hartwig, Karin M. Hoffmeister
Manuscript, 2006

Role of GPIbα clustering and N-linked carbohydrates in the clearance of refrigerated platelets.

Emma Josefsson, Department of Rheumatology and Inflammation Research, Institute of Medicine, The Sahlgrenska Academy, Göteborg University, Göteborg, Sweden; Division of Hematology, Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

The thesis focuses on understanding the mechanisms by which: **1)** the macrophage α_M-subunit recognizes βN-acetylglucosamine (βGlcNAc) residues on the von Willebrand factor receptor complex ($(GPIb_{\alpha,\beta}/IX)_2V$ or vWfR) on refrigerated platelets and **2)** refrigeration changes vWfR to elicit recognition through $\alpha_M\beta_2$. Until recently, the only well-established mechanisms affecting platelet survival were antibody-mediated platelet clearance, consumption of platelets by coagulation reactions, and loss due to massive bleeding. An effort to address a practical problem, how to refrigerate platelets for transfusion, led us to define a previously unsuspected platelet clearance mechanism. We found that (1) macrophages recognize βGlcNAc residues of N-linked glycans on clustered GPIbα subunits following short-term refrigeration (2 h) of platelets in the absence of plasma and (2) phagocytosis and clearance are mediated by the $\alpha_M\beta_2$ integrin receptor of macrophages. Galactosylation of GPIbα blocks ingestion by the macrophage $\alpha_M\beta_2$ and allows short-term refrigerated murine platelets to circulate but does not prevent the removal of platelets stored long-term in plasma.

Work detailed in this thesis demonstrates that the ingestion of short-term refrigerated platelets is dependent on the α_M lectin-domain, not the I-domain which is involved in the recognition of most $\alpha_M\beta_2$ ligands. To address this question, CHO cells were directed to express different α_M/α_x receptor subunit chimeras and the relative contribution of α_M-subdomains to platelet ingestion evaluated in these cells. Critically, the recognition and ingestion of refrigerated platelets by CHO cells occurs only when the α_M-subunits contain the α_M lectin-subdomain. The I- or cation binding subdomains of the α_M-subunit are not required. Soluble recombinant α_M lectin-domain, but not a soluble α_M I-domain, also inhibited the phagocytosis of refrigerated platelets by differentiated macrophages and Sf9 cells expressing solely recombinant α_M lectin-domain constructs bound refrigerated platelets. We conclude, therefore, that refrigeration exposes N-glycan βGlcNAc residues on vWfR which are recognized by the lectin-domain of $\alpha_M\beta_2$ to initiate platelet clearance.

Next, the relationship between vWfR clustering/conformational changes and refrigeration was investigated. Clustering of vWfR is detectable by fluorescent resonance energy transfer (FRET) measured by flow cytometry. Refrigeration of platelets for 24 h markedly increases the FRET efficiency between GPIbα and GPV subunits, whereas the FRET between GPIbα and α_{IIb} is unaltered. We conclude that vWfR aggregation begins immediately following refrigeration but becomes maximal only after extended refrigeration. A panel of monoclonal antibodies (mAbs) that recognize different vWfR subunits was employed to further probe for structural changes. We found that certain epitopes on GPIbα become cryptic as platelets are refrigerated, possibly due to clustering of the vWfR complex, and that the rate of epitope sequestration due to clustering is slowed in the presence of plasma. Changes in binding efficacy of the mAbs are not caused by the loss of GPIbα from the platelet surface as determined by immunoblotting of total GPIbα. Some vWf binding in cold plasma was detected that may influence the binding of mAbs which bind to GPIbα near its vWf binding site. These further changes in vWfR in platelets refrigerated long-term in plasma may be related to the additional phagocytic mechanisms involved in their removal.

Key words: Platelets, GPIbα, vWfR, $\alpha_M\beta_2$, phagocytosis, refrigerated platelets.

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