

# Dual roles for hepatic lectin receptors in the clearance of chilled platelets

Viktorija Rumjantseva

Institute of Biomedicine, Department of Clinical Chemistry and Transfusion Medicine, University of Gothenburg, Göteborg, Sweden; Department of Translational Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, USA

Platelets for transfusion are stored at room temperature because refrigerated platelets are cleared rapidly from circulation. Room temperature storage causes major inconveniences for blood banks and medical risks for recipients. To address this clinically important problem a macrophage-dependent clearance mechanism was defined for platelets refrigerated < 4 h in buffer. Short-term platelet cooling (< 4 h) leads to exposure of  $\beta$ GlcNAc residues on GPIIb $\alpha$ . Exposed  $\beta$ GlcNAc are recognized by lectin domains on hepatic macrophages  $\alpha_M\beta_2$ , which leads to increased clearance. Capping of exposed  $\beta$ GlcNAc by galactosylation using endogenous galactosyl transferases prevented clearance of short-term cooled platelets. In a follow up clinical study, galactosylation did not improve the circulation of human platelets stored in plasma at 4°C for > 48 h (**Paper 1**). To investigate this different outcome we developed a mouse model for studies of storage of platelet-rich plasma (PRP) at 4°C for 48 h. *In vitro* characterization of the 4°C stored mouse platelets showed similar changes as measured on human platelets after 48 h of refrigeration. Importantly, using the mouse model we demonstrated that galactosylation has no effect on the survival of > 48 h long-term refrigerated mouse platelets (**Paper 1**).

Using the mouse platelet storage model we dissected the clearance mechanism of platelets stored for > 48 h in plasma at 4°C, showing that 48 h refrigerated platelets are removed by an additional macrophage-independent mechanism, i.e. by the hepatic galactose-dependent lectin the Ashwell-Morell Receptor (AMR) (**Paper 2**). This conclusion was based on the following evidence: (a) macrophage depletion in mice dramatically improved the circulation of 4 h chilled platelets, but not clearance of 48 h refrigerated platelets; (b) streptavidin-POD staining revealed the localization of long-term refrigerated biotinylated platelets in hepatocytes; (c) 48 h refrigeration tremendously increased the binding of the  $\beta$ galactose-recognizing lectin RCA-I to platelets; (d) KO mice lacking *Asgpr-1* or *Asgpr-2* subunits of the AMR supported 48 h refrigerated platelet circulation; (e) co-injection of asialofetuin, a competitive inhibitor of the AMR, restored the survival of 48 h refrigerated platelets but not of isolated platelets stored for <4 h; (f) ingestion of fluorescently labeled long-term refrigerated platelets in a hepatocyte culture was prevented by asialofetuin. Circulation of 48 h platelets was markedly improved by removal of the N-terminal domain of GPIIb $\alpha$  using O-sialopeptidase (**Paper 2**).

Platelets from mice lacking the sialyltransferase ST3GalIV were used to investigate the increased clearance of platelets with decreased sialylation. We demonstrated that platelets with increased galactose exposure due to the lack of ST3GalIV expression are also removed by a macrophage-independent mechanism, through AMRs on hepatocytes (**Paper 3**).

In conclusion, our results defined a new hepatic-based clearance mechanism for desialylated platelets, representing the first example of blood cell removal by a non-myeloid cell.

**Keywords:** platelet storage, Asialoglycoprotein receptor, GPIIb $\alpha$ ,  $\alpha_M\beta_2$ , transfusion, clearance

ISBN 978-91-628-7943-3

# Dual roles for hepatic lectin receptors in the clearance of chilled platelets

AKADEMISK AVHANDLING

som för avläggande av medicine doktorexamen vid Göteborgs universitet kommer att offentlig försvaras i Förmaket, Sahlgrenska Universitetssjukhuset, Göteborg, torsdag den 19 november 2009, kl 13.00

av

**Viktoria Rumjantseva**

Fakultetsopponent: Professor, Överläkare Martin L Olsson, Sektionen för hematology och transfusionsmedicin Lunds universitet, Lund, Sweden

Avhandling baseras på följande delarbeten:

## **I. Galactosylation does not prevent the rapid clearance of long-term, 4°C-stored platelets**

Hans H. Wandall, Karin M. Hoffmeister, Anne Louise Sørensen, **Viktoria Rumjantseva**, Henrik Clausen, John H. Hartwig, and Sherrill J. Slichter  
Blood, Mar 2008; 111: 3249 - 3256.

## **II. Dual roles for hepatic lectin receptors in the clearance of chilled platelets.**

**Viktoria Rumjantseva**, Prabhjit K. Grewal, Hans H. Wandall, Emma C. Josefsson, Anne Louise Sørensen, Göran Larson, Jamey D. Marth, John H. Hartwig, Karin M. Hoffmeister.  
Nature Medicine, 2009; DOI 10.1038/nm.2030

## **III. Role of sialic acid for platelet life span: exposure of $\beta$ -galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes**

Anne Louise Sørensen, **Viktoria Rumjantseva**, Sara Nayeb-Hashemi, Henrik Clausen, John H. Hartwig, Hans H. Wandall, and Karin M. Hoffmeister.  
Blood, Aug 2009; 114: 1645 - 1654.