Engagement of αIIbβ3 (GPIIb/IIIa) with αvβ3 mediates interaction of melanoma cells with platelets – a connection to hematogeneous metastasis

Anke S. Lonsdorf[1], Björn F. Krämer[2], Manuela Fahrleitner[2], Tanja Schönberger[2], Stephan Gnerlich[2], Sabine Ring[1], Sarah Gehring[2], Stefan W. Schneider[3], Michael J. Kruhlak[4], Sven G. Meuth[5], Bernhard Nieswandt[6], Meinrad Gawaz[2], Alexander H. Enk[1], Harald F. Langer[2].

[1] Department of Dermatology, University Hospital, Ruprecht-Karls University Heidelberg, Germany
[2] Department of Cardiovascular Medicine, University Hospital, Eberhard Karls-University Tübingen, Germany
[3] Experimental Dermatology and Department of Dermatology, Ruprecht-Karls University Heidelberg and Medical Faculty Mannheim, Germany
[4] Experimental Immunology Branch, Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD, USA
[5] Neurological Clinic - Inflammatory Disorders of the Nervous system and Neurooncology / Institute of Physiology, University of Münster, Germany
[6] Chair of Vascular Medicine and Rudolf Virchow Center, DFG Research Center for Experimental Biomedicine, University of Würzburg, Germany

Running title: Platelets and melanoma metastasis

Correspondence to:

Anke S. Lonsdorf, Department of Dermatology, University Hospital, Ruprecht-Karls University Heidelberg, Voßstrasse11, 69115 Heidelberg, Germany.
Phone: +49 (0)6221 – 566364; Fax: +49 (0)6221–561617. E-Mail: anke.lonsdorf@med.uni-heidelberg.de
or
Harald F. Langer, Department of Cardiovascular Medicine, University Hospital, Eberhard Karls-University Tübingen, Otfried-Müller Str.10, 72076 Tübingen, Germany.
Phone: +49-7071-29-82887; Fax: +49-7071-29-5040. E-mail: harald.langer@med.uni-tuebingen.de

Supplemental figure 1
(A, B) Murine B16 cells ($4x10^5$ in 200µl PBS/ animal) were injected i.v. into C57BL/6 mice. After 60min anesthetized animals where transcardially perfused with 20ml PBS to remove circulating platelets and lungs were explanted, embedded into OTC (Tissue Tek) mounting medium and flash frozen at -80°C. 6µm tissue sections were stained for (A) endothelial cells (CD31, green), platelets (GPIIb/IIIa, red) and cellular nuclei (DAPI, blue) or (B) with isotype control IgG and analyzed by immunofluorescence microscopy. Scale bar 100 µm.

Supplemental figure 2
Adhesion of B16 cells in the presence or absence of human platelets ($1x10^8$ /ml) immobilized on collagen (10µg/ml). The presence of immobilized human platelets results in significantly increased adhesion of human melanoma cells (MV3). The mean $\pm$ S.E.M. (n= 3) is shown. *P<0.05 as compared to collagen control.

Supplemental figure 3
Efficient platelet depletion was achieved by intraperitoneal injection of rabbit anti-mouse platelet serum as previously described (1). (A) Analysis of whole blood 24h post injection revealed a platelet depletion efficiency of over 97%. *P<0.05 as compared to control serum. (B) The number of circulating total leukocytes remained unaltered by the treatment with rabbit anti-mouse platelet serum.

Supplemental figure 4
In a control experiment to exclude thrombotic occlusion of the intestinal vessels which may have affected melanoma cell adhesion during intravital videomicroscopy, animals were treated as described in figure 2C and 2D. Instead of staining melanoma cells with DCF, rhodamin-6G was injected to stain any potential forming thrombus, which might have altered blood flow and influenced melanoma cell adhesion (left and middle panel). As a positive control, we treated mice locally with FeCl3 (right panel) to induce extensive thrombus formation (white arrows).

Supplemental figure 5
Principally, experiments were conducted as described in figure 1B. Adherent platelets were incubated with a PE- conjugated rat anti-mouse P-Selectin antibody. Subsequently, platelets were removed from the well using trypsin, washed with PBS and analyzed by flow cytometry. As a negative control, platelets were left in solution for incubation with the antibody and left in solution and additionally "cooled" using PGI2 (2). Platelets were subsequently analyzed for P-Selektin expression by flow cytometry analysis.

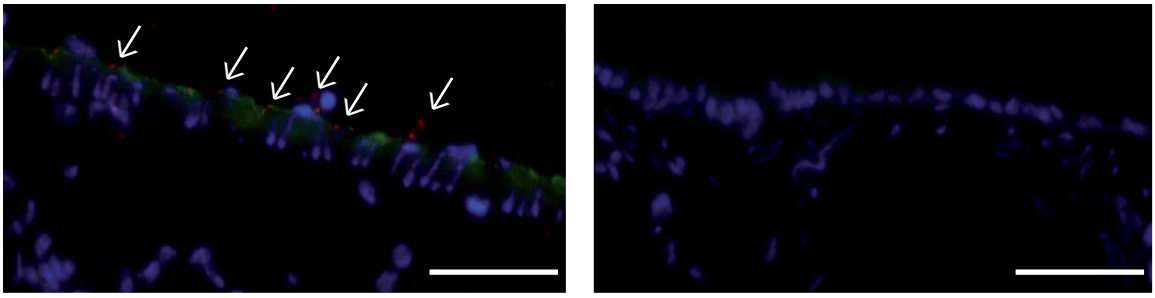
Supplemental figure 6
(A, B) Experiments were conducted as described in figure 3A and 3C. To exclude a difference in the number of adherent platelets by the blockade of integrin binding sites - and as a consequence reduction in melanoma cell adhesion - the number of adherent platelets at the end of the experiment was assessed using an automated whole blood analyzer (Sysmex Se 9000, Kobe, Japan). No significant difference was observed after treatment with RGD protein (A), anti-GPIIb/IIIa antibody (B) or respective controls.

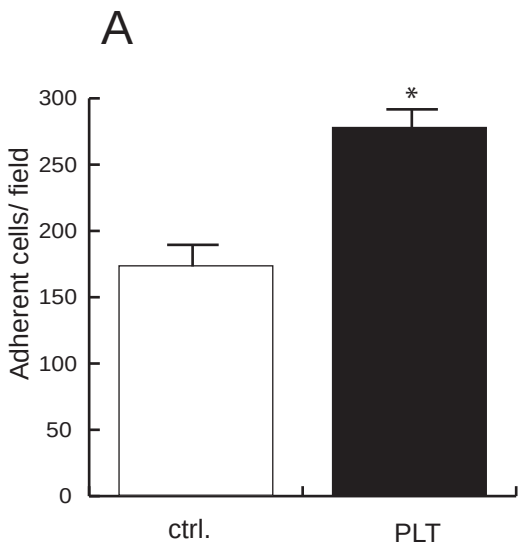
References:

1. Carvalho-Tavares, J., Hickey, M. J., Hutchison, J., Michaud, J., Sutcliffe, I. T., and Kubes, P. (2000) *Circ.Res.* **87,** 1141-1148.

2. Junt, T., Schulze H., Chen Z., Massberg S., Goerge T., Krueger A., Wagner DD., Graf T., Italiano JE Jr., Shivdasani RA., von Andrian UH. (2007) *Science.* **317,** 1167-1170.

A

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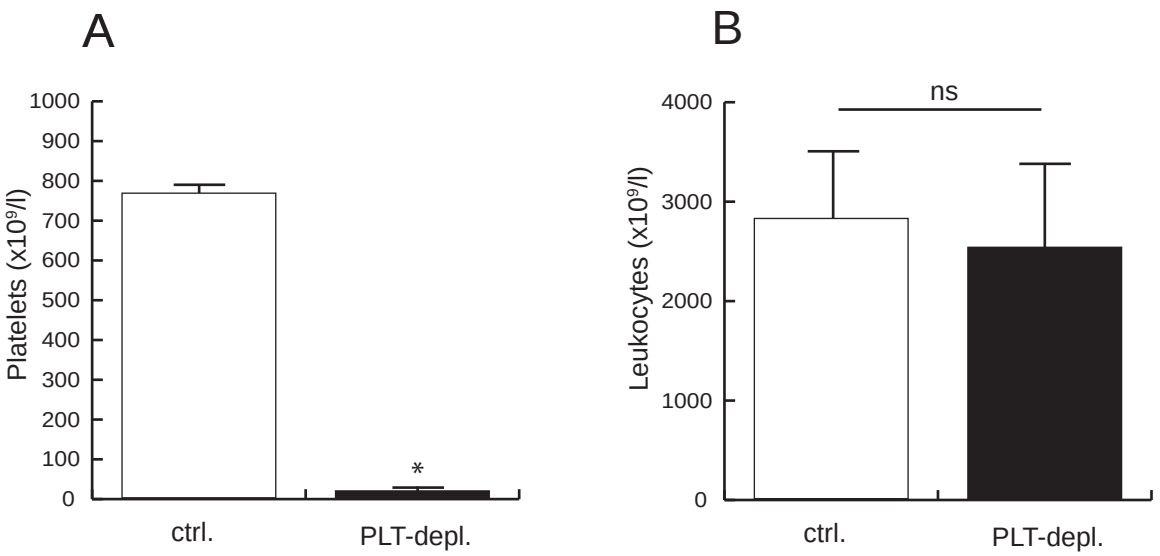
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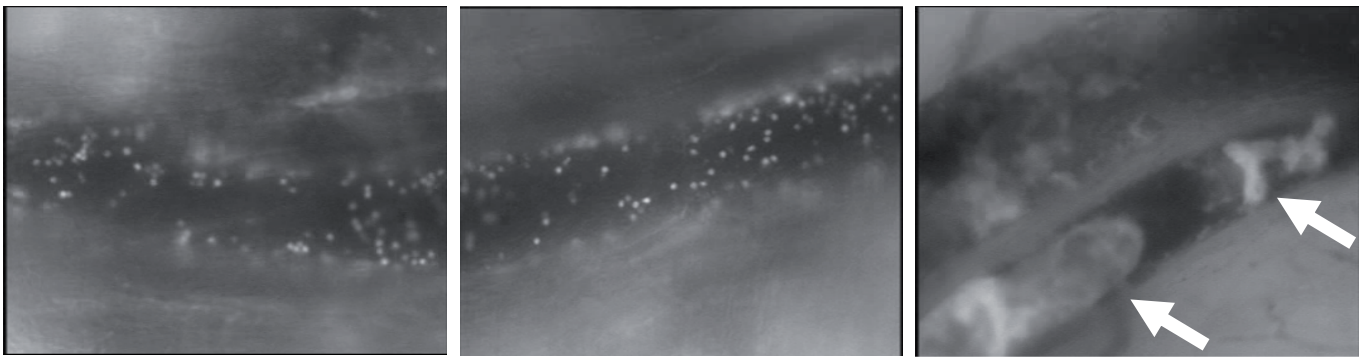
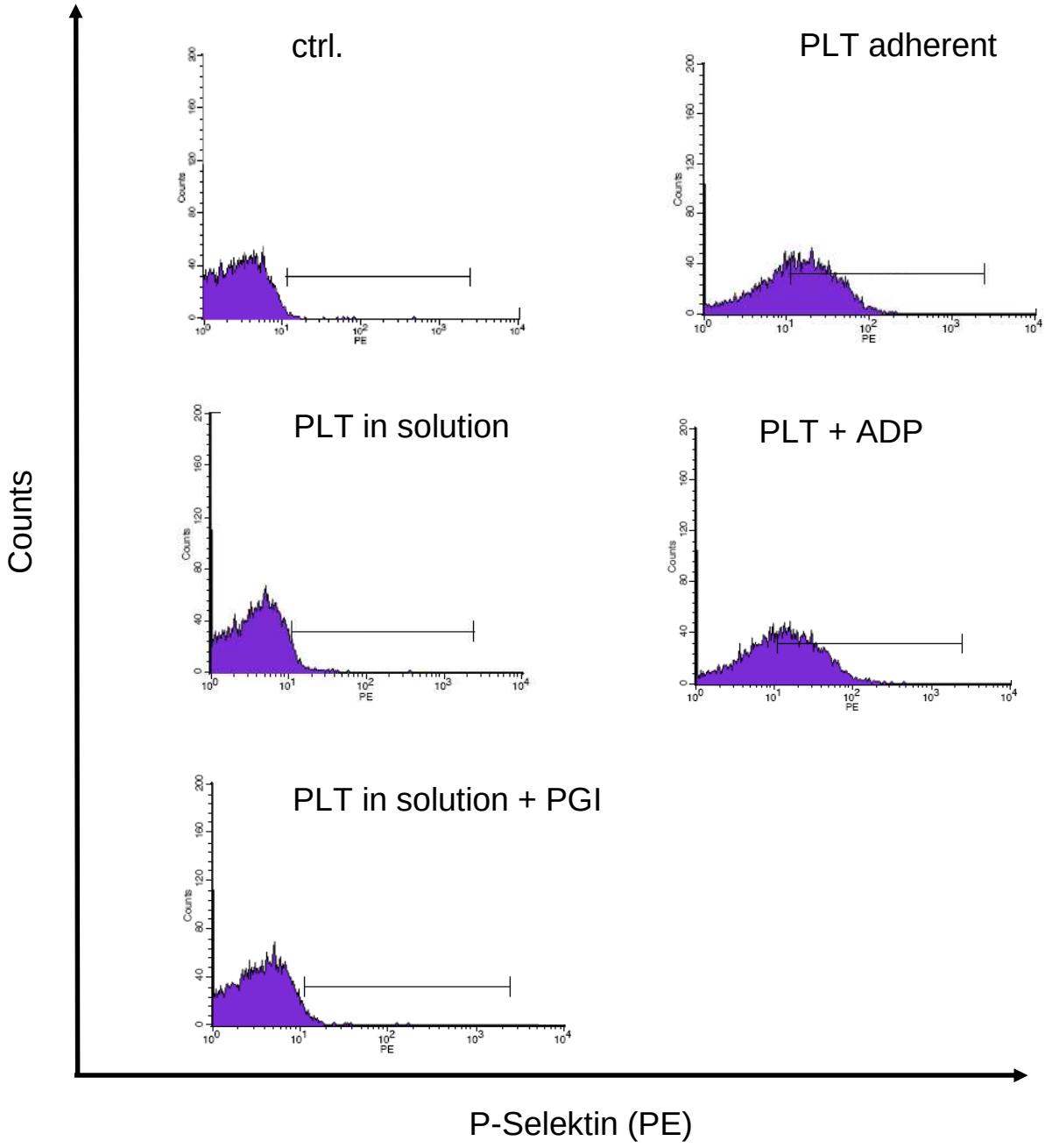


ctrl. PLT-depletion FeCl3 induced thrombus


ctrl.
PLT adherent
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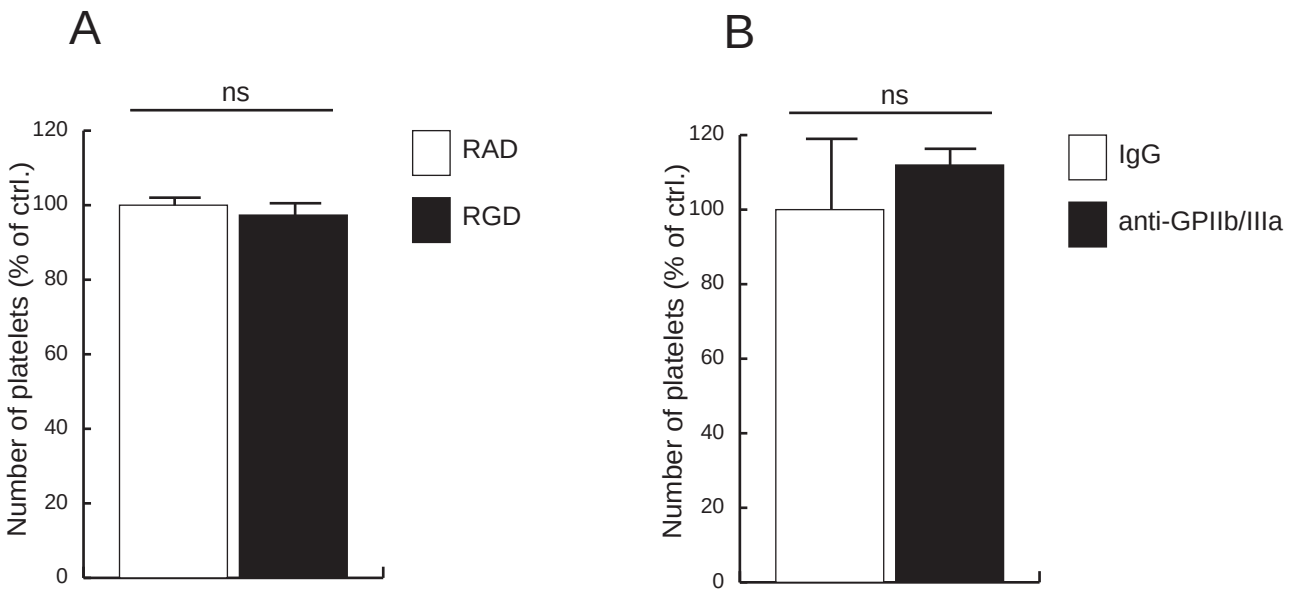
PLT + ADP
PLT in solution + PGI
Counts
P-Selektin (PE)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