Platelet Microparticles
A Transcellular Delivery System for RANTES-Promoting Monocyte Recruitment on Endothelium

Sebastian F. Mause, Philipp von Hundelshausen, Alma Zernecke, Rory R. Koenen, Christian Weber

Objective—Platelet activation mediates multiple cellular responses, including secretion of chemokines such as RANTES, and formation of platelet microparticles (PMPs). We studied the role of PMPs in delivering RANTES and promoting monocyte recruitment.

Methods and Results—Here we show that PMPs contain substantial amounts of RANTES and deposit RANTES on activated endothelium or murine atherosclerotic carotid arteries. RANTES deposition is facilitated by flow conditions and more efficient than that conferred by PMP supernatants. Interactions of PMPs with activated endothelium in flow were mostly characterized by rolling. RANTES deposition showed a diffuse distribution pattern and was rarely colocalized with firmly adherent PMPs, substantiating that RANTES deposition occurs during transient interactions. Importantly, preperfusion with PMPs enhanced monocyte arrest on activated endothelium or atherosclerotic carotid arteries, which could be inhibited by a blocking antibody or a RANTES receptor antagonist. Blockade or deficiency of PMP-expressed adhesion receptors demonstrated differential requirement of P-selectin, glycoprotein Ib (GPIb), GPIIb/IIa, and JAM-A for PMP interactions with endothelium, PMP-dependent RANTES deposition, and subsequent monocyte arrest.

Conclusion—Circulating PMPs may serve as a finely tuned transcellular delivery system for RANTES, triggering monocyte arrest to inflamed and atherosclerotic endothelium, introducing a novel mechanism for platelet-dependent monocyte recruitment in inflammation and atherosclerosis. (Arterioscler Thromb Vasc Biol. 2005;25:1-7.)

Key Words: atherosclerosis ■ adhesion molecules ■ platelets ■ microparticles ■ chemokines

Platelets are versatile blood cells participating in thrombosis and hemostasis but are increasingly attributed with proinflammatory properties.1 As a rich source for cytokines and chemokines, platelets contribute to the regulation of critical steps in the cascade process of integrin-dependent leukocyte recruitment.2 Platelet chemokines and precursors, comprising CXC chemokines such as platelet factor 4 (PF4) or β-thromboglobulins, and CC chemokines such as macrophage inflammatory protein-1β or RANTES, are stored in α-granules and can be rapidly released after platelet activation.3 RANTES has been detected on the luminal surface of atherosclerotic murine and human carotid arteries;4,5 or neointimal lesions after arterial injury and can be deposited on inflamed or atherosclerotic endothelium by activated platelets, thereby triggering monocyte recruitment in flow.4,6,7 Hence, immobilization of RANTES by platelets appears to be involved in the exacerbation of lesion formation inapolipoprotein E−/− (apoE−/−) mice injected with activated platelets.7 Conversely, injection of P-selectin–deficient platelets with an impaired capability to deposit RANTES6,7 did not promote native atherosclerosis,7 and mice deficient in platelet P-selectin were protected from atherosclerosis and neointimal hyperplasia.8,9 Treatment with the antagonist Met-RANTES reduced leukocyte infiltration and progression of atherosclerosis in a hyperlipidemic mouse model,4,5 substantiating the role of RANTES in vivo.

Activation of platelets results in the formation of platelet microparticles (PMPs). These membrane vesicles, ranging in size from 0.1 to 1.0 μm, are shed by platelets after stimulation with physiological agonists such as thrombin or collagen10,11 or in response to high shear stress (eg, in severe stenosis).12 An array of platelet-derived adhesion and chemokine receptors, such as P-selectin, platelet glycoprotein Ib/IIa (GPIIb/IIa), GPIb, and CXCR4,10,24 is present on the surface of PMPs. In addition to procoagulant functions,10 several studies suggest a role of PMPs in inflammatory processes during vascular pathogenesis. Elevated levels of circulating PMPs have been described in patients with arteriosclerosis, acute vascular syndromes, or diabetes mellitus.11 One mechanism by which PMPs enhance adhesiveness of inflammatory target cells is based on the transfer of arachidonic acid, resulting in an upregulation of integrins and adhesion molecules on...
monocytes or endothelial cells. Alternatively, PMPs may transfer platelet-derived adhesion receptors (e.g., GPIb) to hematopoietic cells and increase their endothelial homing.

PMPs may also activate endothelial cells via interleukin-1β (IL-1β), induce cytokine production by monocytes and endothelial cells, and increase leukocyte aggregation and recruitment via P-selectin-mediated interactions.

In this study, we tested whether PMPs may act as transcellular delivery modules for platelet-derived RANTES, resulting in RANTES immobilization and thus promoting enhanced recruitment of monocytes on inflamed or atherosclerotic endothelium.

**Methods**

**Cell Culture and Reagents**

Human microvascular endothelial cells (HMVECs; PromoCell) and monocytic Mono Mac 6 cells were cultured as described. The RANTES receptor antagonist Met-RANTES was provided by A. Proudfoot (Serono Pharmaceutical Research Institute). Soluble JAM-A-Fc fusion proteins (sJAM-A-Fc) were generated as described. All other reagents were from Sigma-Aldrich.

**Generation and Isolation of PMPs**

Platelets were isolated from platelet-rich plasma of healthy donors or a patient with Glanzmann’s thrombasthenia, washed in Krebs-Ringer, resuspended in HHMC at 3x10^6/mL, and activated with 1 U/mL thrombin and 8 ng/mL collagen for 30 minutes at 37°C. After 1250g centrifugation, supernatants containing PMPs were passed through 0.8-μm filters and pelleted at 20,000g for 20 minutes. PMPs were resuspended in a volume identical to supernatants, and PMP quantities in each assay were adjusted for protein content, as analyzed by Bio-Rad assay. Purity was >99%, as analyzed by flow cytometry (GPIb staining and light scatter).

**ELISA, Flow Cytometry, and Immunoblotting**

Concentrations of human RANTES were determined using the DuoSet ELISA (R & D Systems) with a detection limit of 6.25 pg/mL. PMPs, PMP supernatants, and supernatants of activated or nonactivated platelets were lysed in radioimmunoprecipitation assay buffer. To detect RANTES, P-selectin, GPIb/IIa, GPIb, JAM-A, and CX3CR1 on the surface, PMPs were fixed in 3.7% formaldehyde and reacted with primary antibodies (monoclonal antibody [mAb] VL-1, Caltag; mAb 9E1, R & D Systems; 6E3, Lilly; mAb S22, Beckman-Coulter; polyclonal JAM-A Ab, polyclonal CX3CR1 Ab, Torrey Pines Biologs) or isotype controls. Antibody binding was detected with fluorescein isothiocyanate (FITC)–conjugated secondary antibodies and analyzed by flow cytometry using a FACScalibur (Becton Dickinson). Equal amounts of protein from PMP lysates were separated by SDS-PAGE and reacted with primary antibodies to RANTES, PF4, or P-selectin, horseradish peroxidase–conjugated antibody, and super-signal ECL solution (Pierce). Surface RANTES, as determined by specific mean fluorescence intensity and normalized to P-selectin, was related to its content in total PMP lysates, as quantified by densitometry of immunoblots.

**Laminar Flow Assays**

Laminar flow assays for monocyte arrest have been described. Confluent HMVECs were activated with IL-1β (10 ng/mL) for 12 hours and integrated in a flow chamber. PMPs, PMP supernatants, or assay buffer with or without VL-1 mAb (10 μg/mL) were perfused at 1.5 dyne/cm² or incubated in stasis at 37°C for 15 minutes. For inhibition studies, PMPs were pretreated with blocking antibodies to P-selectin, GPIb/IIa, GPIb, CX3CR1, isotype controls (all 20 μg/mL), or sJAM-A-Fc (10 μg/mL), and washed. To rule out direct interactions of monocytes with PMPs or soluble RANTES in PMP supernatants, assay buffer was perfused for 2 minutes before sequentially perfusing Mono Mac 6 cells pretreated with or without Met-RANTES (1 μg/mL) at 1.5 dyne/cm². Firmly adherent Mono Mac 6 cells were counted in multiple fields recorded by video microscopy. Intra-assay and interassay variability for control monocytes were 9.5% and 14.8%, respectively. Incubation of HMVECs with PMP-associated or PMP-inducible chemokines (RANTES or monocyte chemoattractant protein-1 [MCP-1]) for 15 minutes did not affect P-selectin or intercellular adhesion molecule-1 expression, nor did MCP-1 blockade interfere with monocyte arrest, excluding effects related to MCP-1 induction or endothelial adhesion molecules (data not shown). The velocity of PMP rolling was determined by measuring the distance of CM–1,1-dioleoyl-sn-glycerol-3-phosphoethanolamine (DiI; Molecular Probes)–labeled PMPs during an exposure of 100 ms. Rolling was defined as transient P–endothelium interactions with a velocity <500 μm/s. Shear-resistant adhesion of PMPs was quantified by analyzing the average Di-staining intensity per individual endothelial cell after subtracting background.

**Immunofluorescence**

Detection of surface-bound RANTES has been described. After perfusion or static incubation, activated HMVECs were fixed with 2% paraformaldehyde (PFA) and blocked with 2% BSA. Cells were reacted with VL-1 or isotype control and FITC-conjugated secondary antibody. RANTES immobilized on the endothelial surface was quantified by determination of the average FITC-fluorescence intensity of individual endothelial cells (>40 cells per condition) after subtracting background fluorescence using AnalySIS software (Soft- Imaging Systems). Intra-assay and interassay variability for control PMPs was 7.6% and 15.8%, respectively. Adhesion of PMPs was quantified and correlated with RANTES staining intensity.

**Ex Vivo Perfusion and Immunofluorescence of Murine Carotid Arteries**

Animal studies were approved by local authorities. Carotid arteries from 6- to 8-week-old apoe−/− mice fed a high-cholesterol diet were prepared and isolated for ex vivo perfusion. After preperfusion with PMPs or assay buffer for 20 minutes, Mono Mac 6 cells (0.5x10^6/mL) labeled with calcine/AM (Molecular Probes) and preperfused with or without Met-RANTES (1 μg/mL) were perfused for 8 minutes. Accumulation of monocytes was recorded using stroboscopic epifluorescence illumination microscopy. Intra-assay and interassay variability for control monocytes was 11.3% and 16.0%, respectively. For immunofluorescence, ex vivo perfused carotid arteries were fixed in situ by perfusion with 4% PFA, paraformaldehyde, and cut into serial 5-μm sections. Slides were reacted with VL-1 or isotype control and a FITC-conjugated secondary antibody.

**Scanning Electron Microscopy**

Activated HMVECs or carotid arteries perfused with PMPs were fixed with 3% glutaraldehyde (GA) or 2% GA1/1% PFA. Unreacted aldehydes were quenched with glycine, and unspecific binding was blocked. Specimens were reacted with VL-1 and secondary IgG conjugated with 30-nm gold or carbon. Scanning electron microscopy (SeEM) images (FEI/Philips ESEM XL30 FEG) were acquired in secondary electron detection, back-scattered electron (BSE) detection, or mixed detection mode.

**Statistical Analysis**

Data are expressed as mean±SEM. Statistical analysis was performed with Prism 4 software (Graph Pad) using a tailed Student t test or 1-way ANOVA with Newman–Keuls post test. Differences with P<0.05 were considered significant. The number of independent experiments is stated in the legends.
Results

Activation of platelets results in the release of α-granular products, including RANTES, and in the formation of PMPs. To study whether RANTES is transferred into PMPs during vesiculation, we determined RANTES levels in supernatants of nonactivated and activated platelets (nonact./act. platelet sup), in PMPs, and PMP supernatants (PMP sup). Data represent mean±SEM (n=3). B, The presence of RANTES on the surface of PMPs was analyzed by flow cytometry. Representative histograms for RANTES staining (open) or isotype control (filled) are shown (n=3). C, Representative immunoblots for RANTES, P-selectin, and PF4 in nonactivated platelets (1), PMPs (2), and positive control (3; n=3). D, Immunofluorescence of IL-1β-activated HMVECs perfused with PMPs (left) or assay buffer (right). Bar=20 μm. Shown are representative images (n=5). E, Activated HMVECs were perfused or incubated in stasis with PMPs, PMP supernatants, or assay buffer (control), and RANTES deposition was quantified by immunofluorescence. Data are expressed as percentage of control and represent mean±SEM (n=4). *P<0.01 vs PMPs in flow.

We next analyzed whether the deposition of RANTES by PMPs correlated with their adhesion to the endothelial surface in flow. Whereas shear-resistant adhesion of Dil-labeled PMPs was observed in focal areas of some but not all endothelial cells, RANTES showed a scattered distribution pattern (Figure 2A). Moreover, image analysis did not reveal a correlation between the average staining intensity for RANTES and adherent PMPs per endothelial cell (r=0.01;
ScEM demonstrated that firm adhesion of PMPs to endothelial cells occurred without membrane fusion after 15 minutes (Figure 2B). Immunogold staining for RANTES was only rarely colocalized with adherent PMPs and failed to reveal an enrichment of RANTES in the immediate vicinity of PMPs (Figure 2C). Thus, the flow-fostered deposition of RANTES may be dissociated from firm PMP adhesion and supported by transient interactions with endothelium.

Real-time analysis of the dynamic PMP interactions with activated endothelium revealed that the majority of PMPs did not undergo firm adhesion, whereas many engaged in transient interactions resembling continuous and fast rolling (Figure 3A). Rolling of PMPs was characterized by a velocity of 380±36 μm/s, which is nearly twice as high as that described for platelet rolling. Because interactions of platelets with endothelium are known to involve multiple adhesion and signaling molecules, we explored the mechanistic role of PMP-expressed receptors in endothelial interactions and RANTES delivery.

Figure 3. Differential role of PMP-expressed adhesion receptors in PMP functions. A, Rolling of PMPs. Dil-labeled PMPs were perfused on activated HMVECs, and images were recorded with an exposure time of 100 ms. Bar=50 μm. Shown are representative images (n=4). B, The presence of P-selectin, GPIb, GPIIb/IIIa, JAM-A, and CX3CR1 on the surface of PMP was analyzed by flow cytometry. Representative histograms for staining with specific antibodies (open) or isotype controls (filled) are shown (n=3). C, Dil-labeled PMPs left untreated (control) or pretreated with control or blocking agents were perfused over activated HMVECs. Quantification of PMP rolling (left), shear-resistant PMP adhesion (middle), and PMP-mediated RANTES deposition (right). D, RANTES deposition (left) by PMPs obtained from healthy individuals or a patient with Glanzmann’s thrombasthenia (GT). Dot plot analysis of thrombin/collagen-stimulated PMP formation (right) vs baseline (middle) in thrombasthenic platelets. Data are expressed as percentage of control and represent mean±SEM (n=4). *P<0.01 vs control.
JAM-A can participate in platelet adhesion to endothelium, and the fractalkine–CX3CR1 axis has been involved in platelet activation. We indeed detected JAM-A fused with PMPs or assay buffer (control) and the arrest of Mono Mac 6 cells treated with or without Met-RANTES at 3 PMP surface. JAM-A can participate in platelet adhesion to endothelium, and the fractalkine–CX3CR1 axis has been involved in platelet activation. We indeed detected JAM-A fused with PMPs or assay buffer (control) and the arrest of Mono Mac 6 cells treated with or without Met-RANTES at 3 minutes. Data represent mean±SEM (n=0. 0.01 vs untreated monocytes. *P<0.01 vs PMPs.

Figure 5. Role of PMPs in the context of atherosclerosis. A and B, PMP-mediated RANTES deposition on early atherosclerotic carotid arteries after ex vivo perfusion with PMPs. Representative images of RANTES immunostaining in apoE−/− carotid arteries after ex vivo perfusion with PMPs (A) or assay buffer (B) are shown. Bar=10 μm. C, Representative en face ScEM image (BSE mode) of immunogold labeling for RANTES on the luminal surface of carotid arteries perfused with PMPs. RANTES was not colocalized with single adherent PMPs (circle). Bar=2 μm. D, ScEM image (SE mode) of a PMP adherent to atherosclerotic endothelium in a perfused carotid artery. Adhesion was only sporadically observed. Bar=1 μm. E, Monocyte arrest on early atherosclerotic endothelium in flow. Carotid arteries were preperfused with PMPs or assay buffer (control) and the arrest of Mono Mac 6 cells treated with or without Met-RANTES at 3 μL/min was determined after 8 minutes. Data represent mean±SEM (n=4). *P<0.01 vs untreated monocytes.

Figure 4. Monocyte recruitment on activated endothelium. A, HMVECs were preperfused with PMPs or PMP supernatants treated with or without RANTES mAb or assay buffer (control). Untreated or Met-RANTES–treated monocytes were perfused, and the number of adherent cells was determined. *P<0.01 vs PMPs; #P<0.01 vs PMP supernatant (sup; with untreated monocytes). B, Role of PMP adhesion receptors in PMP-mediated arrest of monocytes. Activated HMVECs were preperfused with assay buffer (control) or with PMPs pretreated with blocking or control agents. Data are expressed as percentage of control and represent mean±SEM (n=0). *P<0.01 vs PMPs.

Figure 3. RANTES deposition and monocyte recruitment by PMPs: role of PMP receptors and GPIIb/IIIa. A, HMVECs were preperfused with PMPs (A) or assay buffer (B) are seen. Bar=5 μm. C, RANTES staining on the luminal surface of carotid arteries after ex vivo perfusion with PMPs (A) or assay buffer (B) are shown. Bar=5 μm. D, ScEM image (SE mode) of a PMP adherent to atherosclerotic endothelium. Representative images of RANTES immunostaining in apoE−/− carotid arteries after ex vivo perfusion with PMPs (A) or assay buffer (B) are shown. Bar=10 μm. C, Representative en face ScEM image (BSE mode) of immunogold labeling for RANTES on the luminal surface of carotid arteries perfused with PMPs. RANTES was not colocalized with single adherent PMPs (circle). Bar=2 μm. D, ScEM image (SE mode) of a PMP adherent to atherosclerotic endothelium in a perfused carotid artery. Adhesion was only sporadically observed. Bar=1 μm. E, Monocyte arrest on early atherosclerotic endothelium in flow. Carotid arteries were preperfused with PMPs or assay buffer (control) and the arrest of Mono Mac 6 cells treated with or without Met-RANTES at 3 μL/min was determined after 8 minutes. Data represent mean±SEM (n=4). *P<0.01 vs untreated monocytes.
uted on atherosclerotic endothelium of PMP-perfused carotid arteries (Figure 5C) but is not present in buffer-perfused arteries (data not shown). ScEM analysis revealed that firm adhesion of PMPs only sporadically occurred on early atherosclerotic endothelium (Figure 5D), suggesting that RANTES deposition in the context of atherosclerosis largely depends on transient interactions of PMPs. In line with in vitro findings, preperfusion of PMPs increased the arrest of monocytes on atherosclerotic endothelium (Figure 5E). This increase was prevented by pretreatment of monocytes with Met-RANTES. These data indicate that PMP-derived RANTES may be crucial for the atherogenic recruitment of monocytes.

Discussion

The release of RANTES and the transfer of α-granule constituents and cytokines to PMPs has been described after platelet activation.10,15 Here we show that on stimulation of platelets, substantial amounts of RANTES are redistributed to PMPs. Because the extent of RANTES on the PMP surface is limited, the main portion of RANTES associated with PMPs appears to be present within PMPs. RANTES deposition to activated endothelium is favored by flow conditions and is more efficient after perfusion with PMPs than with PMP supernatants. This difference infers that in addition to the quantity of available RANTES, its deposition depends on the mode of delivery.

The diffuse distribution of surface-bound RANTES and the lack of correlation between the extent of PMP adhesion and the intensity of endothelial RANTES staining further indicated that RANTES deposition is crucially supported by transient interactions. Indeed, real-time analysis revealed that PMPs frequently roll, whereas few undergo arrest in focal areas of endothelial cells. The fast rolling of PMPs may allow a higher frequency of interactions with the endothelial surface than firm arrest and may thus represent the preferred mode of RANTES delivery. This notion was substantiated by our findings that a direct colocalization of PMPs and RANTES immobilized on the endothelial surface was rarely observed. Because the majority of adherent PMPs did not show RANTES staining, a fusion of PMPs with endothelial cells, by analogy to transfer of GPIb or CXCR4 by PMPs,24,25 is unlikely to constitute a mechanism for RANTES delivery. Accordingly, adherent PMPs could be documented by ScEM on activated HMECs without signs of incorporation or integration into the endothelial surface after 15 minutes.

Interactions of platelets with endothelium have been described to involve the platelet adhesion receptors P-selectin, GPIIb/IIIa, GPIb, and JAM-A, whereas P-selectin has also been implicated in RANTES deposition by activated platelets.5,22,25 The concept that RANTES deposition is supported by transient PMP interactions with endothelial cells may invoke a role of these molecules as adhesion or signaling receptors in the delivery of RANTES by PMPs. Blocking P-selectin or GPIb reduced rolling, adhesion, and RANTES deposition, whereas blocking GPIIb/IIIa or JAM-A inhibited RANTES deposition but not rolling or adhesion. This infers that transient interactions of PMPs with endothelium are necessary but not sufficient for RANTES delivery, and that outside-in signaling mechanisms involving GPIIb/IIIa and JAM-A may be operative in RANTES release or transfer by PMPs. Similarly, the release of PF4 by platelets can be stimulated by engagement of GPIIb/IIIa and inhibited with c7E3.26 Although RANTES delivery was impaired in PMPs from thrombusthemic platelets deficient in GPIIb/IIIa, the residual activity suggests that a concerted action of PMP receptors is crucial in RANTES deposition. The fact that GPIIb/IIIa was not involved in PMP adhesion may indicate that the critical inside-out signaling machinery may be absent or uncoupled in PMPs. This is in line with findings that CX,CR1, despite promoting platelet adhesion,23 was not relevant in our assays. In addition, outside-in signaling via engagement of JAM-A can trigger platelet activation after mAb cross-linking27 but may also occur after binding endothelial counterparts. Our data suggest that the contribution of P-selectin and GPIb is a prerequisite for PMP-mediated RANTES deposition by enabling transient contact of PMPs with endothelium, whereas precise features of the signals transmitted by PMP receptors to trigger RANTES delivery remain to be elucidated.

Besides RANTES, activated platelets can secrete multiple chemokines.3 For instance, PF4 has been found on atherosclerotic endothelium after perfusion with activated platelets7 and can act in concert with RANTES to synergistically enhance monocyte arrest in flow.28 It is tempting to speculate that these chemokines can also be delivered by PMPs. Indeed, preliminary data indicate that PMPs deposit PF4 on activated endothelium, inferring a cosequestration of platelet chemokines into PMPs (Mause and Weber, unpublished data). Moreover, circulating PMPs may form complexes with monocytes in vivo and thereby enhance monocyte arrest on endothelium, as seen with platelet–monocyte complexes.29 This hypothesis is corroborated by a report that PMPs can mediate leukocyte interactions via P-selectin, supporting their aggregation or accumulation on selectin-bearing surfaces.17 However, such a formation of PMP–monocyte complexes was excluded in our study by sequential perfusion of PMPs and monocytes.

The proinflammatory and proatherogenic potential of PMPs was substantiated by findings that PMPs can deposit RANTES and thereby enhance monocyte recruitment to early atherosclerotic endothelium in carotid arteries of apoE mice. The moderate degree of PMP adhesion and the diffuse RANTES distribution infer that mechanisms for transient PMP interactions and RANTES deposition observed in vitro are also operative in the perfused atherosclerotic carotid artery. Because elevated levels of circulating PMPs have been detected in patients with atherosclerotic diseases,11 these processes may also occur in vivo. By triggering RANTES-dependent monocyte recruitment in an atherosclerotic context, PMPs may not only act as a marker of disease activity but also as important functional modules in the exacerbation of lesion formation, as seen after intermittent injection of activated platelets, given that both processes depend on P-selectin.7,9 Conversely, inhibition of PMP formation or modulation of the PMP functions identified herein may be relevant to abrogate deleterious effects and lead to clinical benefits in vascular disease.
Our data extend the understanding of the function of PMPs in inflammation and atherosclerosis. The participation of PMPs in these processes is based on versatile mechanisms by which PMPs may modulate pivotal monocyte–endothelium interactions involving different elements of a multistep adhesion cascade. These include the induction of β2-integrins on monocytes and the induction of endothelial adhesion molecules by delivery of arachidonic acid, the inflammatory activation of endothelial cells by IL-1β associated with PMPs, and the endothelial transfer of platelet-derived adhesion receptors. As shown herein, the finely controlled deposition of platelet chemokines directly triggering monocyte arrest on endothelium provides direct evidence for a novel function of PMPs in the context of atherogenesis. Thus, elevated levels of PMPs may not solely reflect an epiphenomenon of platelet activation but rather be regarded as an active transcellular delivery system for proinflammatory mediators and platelet receptors. A selective targeting of adhesion events involved in this mechanism (eg, by blocking P-selectin) might be useful in interfering with inflammatory disorders or atherosclerosis accompanied by platelet activation and enhanced PMP generation.

Acknowledgments
This study was supported by Deutsche Forschungsgemeinschaft (WE19132/3, WE19135/1). We thank M. Bovi for expert help with scanning electron microscopy and T. Kogel and M. Roller for excellent technical assistance.

References
9. Rozmyslowicz T, Majka M, Kijowski J, Murphy SL, Conover DO, Poncz M, Rajatczak J, Gaulton GN, Rajatczak MZ. Platelet- and megakaryocyte-derived microparticles transfer CXC4 receptor to CXC4-null cells and make them susceptible to infection by X4-HIV. AIDS. 2003;17:33–42.
12. Naik UP, Ehrlich YH, Kornecki E. Mechanisms of platelet activation by a stimulatory antibody: cross-linking of a novel platelet receptor for CXCR4 on CXCR4-null cells and make them susceptible to infection by X4-HIV. AIDS. 2003;17:33–42.
13. Naik UP, Ehrlich YH, Kornecki E. Mechanisms of platelet activation by a stimulatory antibody: cross-linking of a novel platelet receptor for CXCR4 on CXCR4-null cells and make them susceptible to infection by X4-HIV. AIDS. 2003;17:33–42.
19. Naik UP, Ehrlich YH, Kornecki E. Mechanisms of platelet activation by a stimulatory antibody: cross-linking of a novel platelet receptor for CXCR4 on CXCR4-null cells and make them susceptible to infection by X4-HIV. AIDS. 2003;17:33–42.
20. Naik UP, Ehrlich YH, Kornecki E. Mechanisms of platelet activation by a stimulatory antibody: cross-linking of a novel platelet receptor for CXCR4 on CXCR4-null cells and make them susceptible to infection by X4-HIV. AIDS. 2003;17:33–42.
Platelet Microparticles. A Transcellular Delivery System for RANTES-Promoting Monocyte Recruitment on Endothelium

Sebastian F. Mause, Philipp von Hundelshausen, Alma Zernecke, Rory R. Koenen and Christian Weber

Arterioscler Thromb Vasc Biol. published online May 12, 2005; Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2005/05/12/01.ATV.0000170133.43608.37.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/