Activated, Not Resting, Platelets Increase Leukocyte Rolling in Murine Skin Utilizing a Distinct Set of Adhesion Molecules

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Selectin-mediated tethering and rolling initiates the multi-step process of leukocyte extravasation which is crucial for the formation of an inflammatory infiltrate. We studied the impact of platelets on this process in the skin. Using intravital microscopy, we analyzed platelet interactions with cutaneous post-capillary venules of mouse ears and observed an increase in platelet rolling if platelets were activated (41.6 ± 20.2% vs. 13.1 ± 8.5% rolling of resting platelets). Experiments with P-selectin deficient mice and antibodies blocking either P-selectin, GPIIb/IIa or GPIb showed that rolling depends on platelet PSGL-1 and GPIIb/IIa on one hand, and endothelial P-selectin on the other. Next, formation of platelet-leukocyte aggregates was demonstrated by simultaneous observation of platelets and leukocytes in vivo utilizing a newly developed two-color technique. Aggregates increased overall leukocyte rolling (leukocytes alone: 27.4 ± 11.2%, leukocytes with resting platelets: 25.3 ± 10.2%, leukocytes with activated platelets 38.1 ± 11.8%). To investigate if activated platelets may contribute to the pathogenesis of chronic cutaneous inflammation, platelet P-selectin expression was studied in 8 patients with psoriasis. A correlation between platelet P-selectin expression and disease severity was established. In summary, we show that activated, not resting platelets increase leukocyte rolling in murine skin. This increased rolling is due to the aggregate formation of platelets with leukocytes. We also provide evidence for a potential role of this mechanism in the pathogenesis of chronic inflammatory skin diseases.

Key words: adhesion molecules/homing/inflammation/platelets/psoriasis/skin

J Invest Dermatol 122:830 –836, 2004

Migration of leukocytes from the vascular system to sites of pathogenic exposure is a key event in the process of inflammation and enables the organism to defend itself against infectious microorganisms. The complex process of leukocyte extravasation in response to inflammatory stimuli involves several well-defined steps (Butcher and Picker, 1996; von Andrian and Mackay, 2000). It is initiated by short-lived adhesive interactions of endothelial selectins with leukocyte carbohydrate ligands displayed on glycoprotein scaffolds. These interactions slow down leukocytes, which then roll along the endothelium. In addition to their function in coagulation, platelets are increasingly recognized to be involved in this process, indicating their importance as effector cells of the immune system. Injury leads to activation of the coagulation pathways; it also bears the risk of infection. As a consequence of a triggered coagulation cascade, platelets become activated, leading to morphological changes, release of pro-inflammatory mediators (including platelet factor-4, RANTES, and cytokines) and a change in surface adhesion molecule expression (Michelson et al, 1987; Lumadue et al, 1996).

Additionally, platelets may further contribute to inflammation by mediating leukocyte rolling, as they are capable of binding to them. These platelet-leukocyte aggregates have been shown to roll along the endothelium of murine lymph nodes in a cartwheel-like fashion. The functional relevance of aggregate formation has been demonstrated by reconstituting a delayed-type hypersensitivity reaction (which depends on leukocyte extravasation) in L-selectin-deficient mice, which are unable to mount such responses. Rolling of platelet-leukocyte aggregates in lymph nodes involves platelet P-selectin binding to leukocyte PSGL-1 on the one hand, and endothelial PNAd on the other hand (Diacovo et al, 1996, 1998).

Given the role of platelets in leukocyte extravasation in lymph nodes, we asked whether similar mechanisms might also be involved in cutaneous inflammation. Here we show that activated, not resting, platelets are capable of facilitating leukocyte rolling in cutaneous post-capillary venules. Compared to lymph nodes however, a different set of adhesion molecules is involved in the skin compartment. Finally, correlation between platelet activation and the severity of psoriasis, a chronic inflammatory skin disease, also suggests a possible role in human pathology.

Abbreviation: PBMC, peripheral blood mononuclear cell

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Results

Platelet rolling in murine skin is influenced by their state of activation Initially, we characterized the interaction of platelets with post-capillary venules in murine skin using intravital microscopy. When resting platelets were injected into mice, 13.1% ± 8.5% of them were slowed down in the mouse ear and displayed a rolling motion along the endothelium. In contrast, when platelets were activated by pre-incubation with TRAP, rolling increased more than 3-fold to 41.6% ± 20.2% (p < 0.001, paired t test, n = 26/8 vessels/mice). The observed increase was not due to changes in blood flow (470 ± 189 μm per s vs 490 ± 190 μm per s; p = n.s.; paired t test) or to residual TRAP in the platelet suspension (data not shown).

Platelet rolling in murine skin involves a distinct set of adhesion molecules different from the lymph node compartment Activation induces profound changes in adhesion molecules expressed on the platelet surface, including upregulation of P-selectin, PSGL-1, and GPⅡb/Ⅲa as well as a decrease in GPⅠb expression (Michelson et al., 1987; Frenette et al., 2000; Leytin et al., 2000). To identify adhesion molecules involved in the rolling of activated platelets along cutaneous microvessels, resting and activated platelets were injected in both wild-type and P-selectin-deficient mice in order to determine if interaction of platelet PSGL-1 with endothelial P-selectin is involved: in wild-type mice, 41.6% ± 20.2% (n = 26/8 vessels/mice) of activated platelets rolled along the endothelium. Rolling was reduced to 20.4% ± 14.8% in P-selectin-deficient mice (p < 0.001, t test, n = 22/8 vessels/P-sel−/− mice), indicating that endothelial P-selectin plays a major role. Activated platelets in P-selectin-deficient mice, however, still rolled more readily compared to resting platelets (20.4% ± 14.8% vs 6.6% ± 6.8%, p < 0.001, paired t test, n = 22/8 vessels/mice). Involvement of endothelial P-selectin in mediating rolling of activated platelets was confirmed in experiments using an anti-P-selectin antibody, resulting in a significant decrease in the rolling of activated platelets (Fig 1a). While a significant decrease with blockade of endothelial P-selectin in rolling was seen, it was not complete indicating that other adhesion molecules are additionally involved.

Platelet GPⅡb/Ⅲa binds via fibrinogen to endothelial ICAM-1 (Simon et al., 1997; Bombeli et al., 1998), which is constitutively expressed in murine skin (Goebeler et al., 1990; Nyberg et al., 1999; Peschen et al., 1999). Thus, an interaction of GPⅡb/Ⅲa with endothelial ICAM-1 is another likely mechanism mediating the rolling of activated platelets in murine skin microvessels. Similar to the experiments using a murine P-selectin antibody, rolling of activated platelets was significantly decreased when a GPⅡb/Ⅲa antibody was present in the circulation. Again, no complete inhibition was observed (Fig 1b).

GPⅠb is another potential P-selectin ligand expressed on platelets (Romo et al., 1999). A 50% reduction in GPⅠb expression, however, induced by platelet activation is known to have no significant impact on platelet rolling in vitro (Michelson et al., 1987; van Zanten et al., 1998). We confirmed these in vitro findings in our in vivo model by injecting an anti-GPⅠb antibody and observing no effect on platelet rolling (Fig 1c).

Activated platelets form aggregates with leukocytes in vitro Having established the molecular mechanism for platelet rolling along cutaneous post-capillary venules, we now addressed the mechanisms of aggregate formation of platelets with leukocytes (de Bruijne-Admiraal et al., 1992). In order to investigate which adhesion molecules are involved in platelet-leukocyte aggregate formation, freshly isolated resting or activated platelets were incubated with PBMC followed by measurement of platelet-PBMC aggregates using flow cytometry. In this in vitro assay, the binding of activated platelets to PBMC was found to be increased compared to resting platelets (p < 0.05, Table I). Addition of neutralizing antibodies against either P-selectin or PSGL-1 completely blocked the activation-induced binding of platelets to leukocytes, while a combination of both antibodies showed no further decrease in platelet binding. Thus,
Table I. In vitro activated platelets bind PBMC depending on platelet P-selectin and leukocyte PSGL-1

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Resting platelets (%)</th>
<th>Activated platelets (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.8 ± 2.9</td>
<td>30.2 ± 5.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mac-1</td>
<td>22.0 ± 2.6</td>
<td>32.7 ± 4.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P-selectin</td>
<td>15.4 ± 3.4</td>
<td>12.7 ± 2.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>PSGL-1</td>
<td>16.9 ± 2.4</td>
<td>15.7 ± 3.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>P-selectin and PSGL-1</td>
<td>15.6 ± 3.4</td>
<td>13.0 ± 2.3</td>
<td>n.s.</td>
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</tbody>
</table>

PBMC were incubated with either resting or activated platelets in the presence of antibodies. The percentage of platelet-PBMC aggregates was assessed by flow cytometry: platelets were identified with an P-CD41 Ab and the number of aggregates was assessed in a leukocyte gate. Activation-induced binding of platelets predominantly depended upon platelet P-selectin and PBMC PSGL-1. p: resting vs activated platelets; n.s.: not significant. Data are presented as mean ± SD from six experiments.

Activated, not resting, platelets increase leukocyte rolling in vivo As activated platelets roll along cutaneous microvessels in vivo and readily form aggregates with leukocytes in vitro, we hypothesized that activated platelets contribute to leukocyte rolling. In order to visualize the interaction of platelets with PBMC, a two-color intravital microscopy setup was developed by staining platelets with the green fluorescent dye CFDA-SE and PBMC with the red fluorescent dye Dil. PBMC were infused into mice either alone, or together with resting or activated platelets. In concordance with our in vitro studies described above, a 3-fold increase in platelet–PBMC aggregate formation was observed when activated platelets were infused (Table II). The majority of these aggregates of activated platelets and PBMC showed a high tendency for rolling, whereas only a minority of the platelet–PBMC aggregates, formed when resting platelets were present, showed transition into a rolling motion (Table II).

The increased platelet–leukocyte aggregate formation and platelet–leukocyte aggregate rolling observed if activated platelets were present in the circulation impacted overall leukocyte rolling. No difference was observed with regard to leukocyte rolling in the presence of resting platelets. In contrast, leukocyte rolling increased significantly when activated platelets were present. This increased tendency for leukocyte rolling was exclusively due to the formation of platelet–leukocyte aggregates, as non-aggregated leukocytes showed no change in rolling frequency. Overall, about one-third of leukocyte rolling was due to the rolling of aggregates when activated platelets were infused (Fig 3). These observations were not influenced by the blood flow since this parameter remained constant, documented by V_free (V_free PBMC: 460 ± 295 μm per s; V_free PBMC + resting platelets: 495 ± 293 μm per s; V_free PBMC + activated platelets: 529 ± 178 μm per s; p = n.s., paired test).

Platelet activation correlates with disease severity in patients with chronic plaque-stage psoriasis Our observations point toward a possible role of activated platelets in cutaneous inflammation. Therefore, we studied platelet activation in eight patients with psoriasis, a chronic inflammatory skin disease, by flow cytometry using P-selectin expression as a marker for activation (Michelson et al, 1987). As shown in Fig 4, platelet P-selectin expression was increased in eight of eight patients investigated prior to initiation of anti-psoriatic therapy. Successful therapy resulted in a decrease in PASI as well as a decrease in platelet

Figure 2

In vitro activated platelets bind PBMC depending on platelet P-selectin and leukocyte PSGL-1. PBMC were incubated with either resting or activated platelets in the presence of antibodies. The percentage of platelet–PBMC aggregates was assessed by flow cytometry. Platelets was identified with an FITC-CD41 Ab and the number of aggregates was assessed in a leukocyte gate. Activation-induced binding of platelets predominately depended upon platelet P-selectin and PBMC PSGL-1. One representative experiment of six is shown. Addition of anti-P-selectin or anti-PSGL-1 Ab completely inhibited the activation-induced binding of platelets to leukocytes. A combination of the two antibodies had no additional effect.
P-selectin expression to a level similar to those measured in healthy controls (Fig 4). Hypercholesterinemia, known to be associated with platelet activation (Tailor et al., 2003), could be excluded as possible interference in our analyses, since included patients showed normal cholesterol values (184.6 ± 43.9 mg per dL before and 194.6 ± 32.3 mg per dL after therapy; p = n.s.; paired t test). Furthermore, P-selectin expression was found to be independent off both platelet count and general inflammation mirrored by the C-reactive protein (data not shown).

**Discussion**

Leukocyte extravasation is mediated by adhesion molecules expressed on leukocytes and endothelial cells in a stepwise process, beginning with rolling of leukocytes along vessel walls (Butcher and Picker, 1996; von Andrian and Mackay, 2000). This transition to a rolling motion is initiated by short-lived interactions between leukocytes and endothelial cells. In addition, at least in lymph nodes, platelets have been shown to participate in this process by forming bridges between leukocytes and the endothelium. Aggregates of platelets and leukocytes are formed in the bloodstream via interactions between platelet P-selectin and leukocyte PSGL-1, which initiates aggregate rolling on endothelial PNAd expressed at specialized vessels in the lymph node (Diacovo et al., 1996, 1998). Here we show that

### Table II. In vivo formation of platelet–PBMC aggregates is increased when platelets are activated

<table>
<thead>
<tr>
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<th>PBMC and resting platelets (%)</th>
<th>PBMC and activated platelets (%)</th>
<th>p (activated vs resting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet–PMBC aggregates/total PBMC</td>
<td>4.7 ± 4.9</td>
<td>17.9 ± 10.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RF aggregates</td>
<td>18.2 ± 38.6</td>
<td>72.7 ± 26.0</td>
<td>= 0.004</td>
</tr>
<tr>
<td>Rolling aggregates/rolling PBMC</td>
<td>4.6 ± 9.6</td>
<td>32.7 ± 20.4</td>
<td>= 0.001</td>
</tr>
</tbody>
</table>

Using two-color intravitral microscopy, the interaction of platelets and PBMC in the bloodstream as well as the interaction of platelet–PBMC aggregates with endothelium was observed. The rolling fraction (RF) of aggregates was calculated by dividing rolling aggregates by total observed aggregates in a vessel. Aggregates of activated platelets with PBMC show a high rolling fraction and constitute one-third of total PBMC rolling. Data are presented as mean ± SD from n = 12/5 vessels/mice.

**Figure 3** Activated platelets mediate rolling of PBMC in murine skin vessels.

Rolling of PBMC and platelets was simultaneously observed in murine post-capillary venules using two-color intravital microscopy. In the same vascular beds, rolling of (i) PBMC, (ii) PBMC with resting platelets, and (iii) PBMC with activated platelets was consecutively assessed. Activated platelets increased overall PBMC rolling due to rolling of platelet–PBMC aggregates. Right column: identical to the column on the left—the light gray column portion represents rolling of PBMC not bound to platelets, whereas the dark gray column corresponds to the rolling of platelet–PBMC aggregates. Data are presented as mean ± SD from n = 12/5 vessels/mice.

**Figure 4** Platelet P-selectin expression is increased in patients with psoriasis.

(a) Severity of psoriasis and (b) platelet P-selectin expression were evaluated in patients before and after successful therapy (mean duration of therapy 27.3 ± 5.6 d). Increased platelet P-selectin expression was observed only in untreated patients, who reached the levels of healthy controls upon disease remission. (c) Furthermore, the severity of psoriasis as measured by PASI correlated to platelet P-selectin expression in patients (r = 0.83, p < 0.001). (d) In healthy controls, platelet P-selectin expression remained constant over a 4-wk observation period (n = 8 patients and 5 controls).
platelets are also capable of rolling along cutaneous post-capillary venules, a phenomenon influenced by their state of activation.

Activation profoundly alters surface expression patterns of adhesion molecules on platelets: P-selectin, PSGL-1, and GPIIb/IIa are upregulated, whereas GPIb expression decreases. In lymph nodes, rolling of platelet–leukocyte aggregates involves platelet P-selectin and leukocyte PSGL-1 as well as endothelial PNA (Kaplanski et al., 1996, 1998). In contrast, we show that similar, but not identical, platelet–PBMC aggregates utilize platelet PSGL-1 and GPIIb/IIa and endothelial P-selectin to roll along post-capillary venules in the skin. Involvement of distinct sets of adhesion molecules in different immunologic compartments is a prerequisite for the extravasation of specific leukocyte subsets to different tissues in health and disease (Butcher and Picker, 1996; von Andrian and Mackay, 2000). These subsets can be characterized at least in part by their respective tissue-specific homing receptor, such as the cutaneous lymphocyte-associated antigen for skin (Picker et al., 1993). Involvement of platelets as bridging compounds between leukocytes and endothelial cells may potentially decrease the specificity of leukocyte recirculation. Since platelet–PBMC aggregate rolling in the skin relies on distinct adhesion molecules, platelet–PBMC aggregates may also display an ability to home to specific sites.

Rolling of activated platelets along skin microvessels may impact the inflammatory process: activated platelets have been shown to increase the expression of endothelial adhesion molecules such as ICAM-1 and cytokine release (MCP-1 and IL-8) from endothelial cells, a process involving adhesion molecules such as ICAM-1 and cytokine release have been shown to increase the expression of endothelial cells (Diacovo et al., 1993). Involvement of platelets as bridging compounds between leukocytes and endothelial cells may potentially decrease the specificity of leukocyte recirculation. Since platelet–PBMC aggregate rolling in the skin relies on distinct adhesion molecules, platelet–PBMC aggregates may also display an ability to home to specific sites.

The functional relevance of platelets in facilitating leukocyte rolling in the skin has yet to be established in human pathology. Evidence for platelet activation, however, is well established in psoriasis, a chronic inflammatory skin disease (Berrettini et al., 1985; Hayashi et al., 1985; Vila et al., 1991). Our data on increased platelet P-selectin expression in patients with psoriasis further support this notion. Interestingly, a correlation was found between the level of P-selectin expression by platelets and disease severity measured by PASI. In seemingly unrelated areas of research, patients with psoriasis were shown to bear an increased risk for cardiovascular disease (McDonald et al., 1973, 1978; Ena et al., 1985; Mallbris et al., 2001). This may well be related to the activated phenotype of platelets in psoriasis.

We conclude that activated platelets support leukocyte rolling in skin microvasculature by two independent mechanisms: (i) rolling of activated platelets along skin vessels provides an environment of pro-inflammatory mediators, and (ii) activated platelets bind leukocytes in the bloodstream, leading to the formation of aggregates that roll along the endothelium and increase overall leukocyte rolling (Fig 5). Hence, platelets must be considered important effector cells of the skin immune system.

Materials and Methods

Platelet isolation Platelets were isolated by drawing blood from donors through a 21 gauge needle, collected in 3 mL tubes containing Na-citrate (Sarstedt, Numbrecht, Germany), and immediately spun at 110 x g for 15 min at 24°C to gain platelet-rich plasma. Platelets were separated from the plasma proteins by gel filtration (Hartwig et al., 1991) through a Sepharose 2B (Pharmacia, Uppsala, Sweden) column. Platelet isolation did not change P-selectin expression as determined by flow cytometry (data not shown). Platelets were then activated using 2.5 mM thrombin receptor activating peptide (TRAP). Activation status was confirmed by measuring expression of P-selectin on platelets using FACS.

Peripheral blood mononuclear cell (PBMC) isolation Human PBMC were prepared by Ficoll–Hypaque density gradient sedimentation (Pharmacia, Uppsala, Sweden) from buffy coats obtained from the Department for Transfusion Medicine of the Johann Wolfgang Goethe-University Frankfurt, and adjusted to 3 x 10^7 cell per mL in AIM V cell culture medium (Life Technologies, Paisley, UK).
Antibodies and reagents For in vitro platelet-leukocyte binding experiments, the following antibodies were used: PE-labeled HP8 antibody (BD Biosciences, Heidelberg, Germany) to identify platelet CD41a, 9E1 (R&D Systems, Wiesbaden, Germany) recognizing CD62P, PL-1 (Immunotech, Hamburg, Germany), a blocking PSGL-1 Ab and a blocking Mac-1 Ab (clone ICRF44, BD Biosciences). Corresponding isotype antibodies served as controls. The following antibodies were used for intravital microscopy: S22 (Immunotech) recognizing the human GPIib antigen, anti-mouse P-selectin Ab (clone RB40.34 from BD Biosciences) and clone S221 (Immunotech), a blocking Ab for human GPIib/IIIa. TRAP was purchased from Bachem (Heidelberg, Germany).

In vitro binding of platelets to PBMC Resting or activated platelets were incubated with isolated PBMC in the presence of antibody (10 µg per mL) for 15 min at room temperature. After incubation, PE-labeled anti CD41a Ab (clone HP8, BD Biosciences) was added for another 15 min to visualize platelets. In each sample, irrelevant Ab of the appropriate isotype were used as controls. Finally 500 µL of CellWASH solution (BD Biosciences) was added and samples were immediately analyzed on a FACScan (BD Biosciences): PBMC were detected in a gate set on lymphocyte-sized cells. The number of platelet–PBMC aggregates was assessed by dividing the red fluorescent cells (PE-labeled platelets) in the leukocyte gate through the number of all leukocytes.

Fluorescent labeling of PBMC and platelets For intravital microscopy experiments, PBMC (3 × 10^7 cell per mL) were incubated with 2.5 µM Vybrant Dil cell-labeling solution (Molecular Probes, Eugene, Oregon) for 20 min at 37°C in the dark. Treated cells were washed, resuspended to 3 × 10^7 cell per mL in AIM V cell culture medium, and used immediately. For single-color fluorescent experiments, platelets were stained with BCECF (0.2 mM, 30 min at RT, Molecular Probes). For two-color fluorescent intravital microscopy, isolated platelets were incubated with 10 µM CFDA-SE (Molecular Probes) for 30 min at 37°C in the dark. Platelets were separated from excess dye by gel filtration as mentioned above.

Mice Male C57BL/6 mice aged 6–10 wk were obtained from Charles River (Sulzfeld, Germany). P-selectin-deficient mice were originally obtained from Jackson Laboratories (Bar Harbor, Maine), and are now bred in our facility. All animal experiments were approved by the governmental administration of Hessen (Darmstadt, Germany).

Intravital microscopy Intravital microscopy of mouse ears was performed in order to observe the rolling of human leukocytes and platelets whose selectins and ligands efficiently interact across this species barrier (Robert et al, 1999). Additionally, E- and P-selectin, which can be detected in psoriatic skin lesions and other chronic-inflammatory skin diseases (e.g., atopic dermatitis and contact dermatitis (Barker, 1995)), are constitutively expressed in normal species barrier (Robert et al, 1999). Additionally, E- and P-selectin, which can be detected in psoriatic skin lesions and other chronic-inflammatory skin diseases (e.g., atopic dermatitis and contact dermatitis (Barker, 1995)), are constitutively expressed in normal skin. Mice were anesthetized by administering a mixture of pentobarbital sodium and atropine sulfate (Santen, New York) and Rompun (Bayer, Leverkusen, Germany), and placed on a homothermic blanket. The right carotid artery was prepared microsurgically and a catheter was inserted downstream into this vessel for injection of cells and antibodies. The left ear of the mouse was gently placed on a microscope slide, covered with glycerin, and a coverslip. For simultaneous observation of two different cell types in the same vascular bed, a novel two-color intravital microscopy system was developed based on a previously described setup (Schon et al, 2002). Vascular architecture and labeled cells were visualized during their passage through vessels under fluorescent epi-illumination using a multiband-filter system (XF 53, Omega Optical, Brattleboro, Vermont). Continuous video recordings of the microcirculation were obtained using a 1/3” DSP 3-CCD camera (DXC-990, Sony, Köln, Germany) mounted on a modified (Baatz et al, 1995) Zeiss microscope (Axiotherm Vario 100 HD, Zeiss, Germany) equipped with a × 10 saltwater immersion objective (Nikon, Düsseldorf, Germany). Images were stored on tape using a Panasonic AG 7355 videocassette recorder. Subsequently, cells and/or antibodies (4 mg per kg b.w.) were injected. With the exception of antibodies, the order of injection was selected randomly. Cell behavior in individual vessel segments was determined by playback of video tapes. Cells were considered non-interacting when they moved at the velocity of the mean blood flow (V_0), whereas detectable lower velocities were defined as rolling. To determine V_0, the mean velocity of at least 15 non-interacting cells was calculated for each vessel.

Patients For analysis of platelet P-selectin expression, blood from eight patients with psoriasis and five healthy controls was collected in 3 mL tubes containing Na-citrate (Sarstedt, Nümbrecht, Germany). After informed consent was obtained. Thereafter, blood was incubated with FITC-labeled anti CD62P antibody (clone AK4) to stain for P-selectin and PE-labeled anti-CD41a antibody (clone HIPI, both from BD Biosciences, Heidelberg, Germany) for identification of platelets according to the manufacturer’s instructions. After incubation for 15 min at room temperature, samples were diluted with CellWASH solution (BD Biosciences) and analyzed on FACScan (BD Biosciences). Severity of psoriasis was assessed using the “psoriasis area and severity index” (PASI) (Papp et al, 2003). Patients were treated independently from this study. The mean duration of treatment was 27 ± 5 days. Experiments were approved by the ethics committee of the J.W. Goethe University (Frankfurt, Germany) and were conducted in accordance with the Declaration of Helsinki.

Statistical analysis Data are presented as mean ± standard deviation if not otherwise indicated. Statistics were performed using SigmaStat (SPSS, Chicago, Illinois). The following tests were used to calculate statistical significance: Pearson product moment correlation to test for correlation, one-way ANOVA to test for differences in rolling, and the paired t test to test for differences in platelet P-selectin expression before and after therapy.

We gratefully acknowledge the excellent technical help by S. Diehl and K. Hardt-Weinelt. This work was supported by grants from the Deutsche Forschungsgesellschaft (Lu877/1-1) to R.J.L. and J.E.S, and from the J.W. Goethe University to R.L.

DOI: 10.1111/j.0022-202X.2004.22318.x

Manuscript received June 3, 2003; revised September 11, 2003; accepted for publication October 1, 2003

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