The Effect of IL-6-Primed Platelets on ADAMTS13-Mediated Clearance of Platelet-Bearing ULVWF and Its Mechanism

Hyun-Jeong Kim  
Yonsei University College of Medicine

Jing-fei Dong  
Bloodworks Research Institute

Yejin Song  
Yonsei University College of Medicine

Hyo-Il Jung  
Yonsei University

Jaewoo Song (labdx@yuhs.ac)  
Yonsei University College of Medicine

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Abstract

Inflammation is an essential contributing factor in the development of thrombosis. Using a microfluidic flow chamber, we investigated how the proinflammatory cytokine interleukin 6 (IL-6) affects the cleavage of platelet-bearing ultra-large VWF (ULVWF) by plasma ADAMTS13. We found that IL-6-treated platelets perfused at arteriolar shear stress significantly enhanced the ULVWF-platelet complex formation on activated endothelial cells and suppressed their clearance by ADAMTS13 under flow conditions. We also detected the phosphorylation of the serine/threonine kinase Akt and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) in platelets treated with IL-6. Treatment of IL-6-primed platelets with either the phosphoinositol-3 kinase (PI3K) inhibitor LY294002 or the mitogen-activated protein kinase kinase (MEK) inhibitor U0126 reduced the ULVWF-platelet complex formation and restored the clearance of the complex by plasma ADAMTS13, compared to IL-6-primed platelets. Furthermore, IL-6 enhanced the phosphorylation of the intracellular adaptor molecule 14-3-3ζ, which regulates VWF binding to the glycoprotein (GP) Ib-IX complex. The 14-3-3 antagonist R18 significantly increased ADAMTS-13 cleavage of ULVWF strings with adherent IL-6-treated platelets. These findings indicate that IL-6 related intracellular signals of platelet is involved in regulating ULVWF-platelet binding and ULVWF cleavage by ADAMTS13.

1. Introduction

VWF enables platelets to adhere to the subendothelial matrix that can resist the pulling force of blood flow, which can be as high as or even higher than shear stress found in arterioles [1, 2]. While platelets initiate hemostasis when they bind the subendothelial matrix, they also bind the strings of VWF multimers freshly released from activated endothelial cells and anchored to the endothelial surface. ADAMTS13 effectively cleaves and thus remove these platelet-bearing VWF strings [3], especially the hyperactive ULVWF from the endothelial surface because platelets on VWF exert a stretching force on VWF to expose the sessile bond in the A2 domain for ADAMTS-13. The importance of this enzymatic activity of ADAMTS13 is well-demonstrated in patients with thrombotic thrombocytopenic purpura, whose ADAMTS13 activity is either suppressed by autoantibodies to the metalloprotease or deficient due to genetic mutations of the ADAMTS13 gene. If the cleavage is delayed, more platelets could be tethered to the VWF strings before cleavage, thus releasing platelet-VWF aggregates that could cause systemic arterial thrombosis.

The clearance of platelet-bearing ULVWF strings can also be affected by inflammation. We have previously shown that IL-6, a universal inflammatory cytokine, significantly suppressed the ADAMTS13 activity for cleaving platelet-bearing ULVWF strings [4]. It was speculated that IL-6 binds and interferes with ADAMTS13 by steric hindrance on the VWF-ADAMTS13 interaction. However, the fact that IL-6 regulates platelet function [5] also raises the possibility that platelets primed for activation by IL-6 affects the cleavage of ULVWF bearing those platelets. Platelets can also regulate VWF cleavage by providing the surface on which VWF and ADAMTS13 interact to form a proteolytic complex [6] or by VWF undergoing conformational changes upon binding to GP Ibα to facilitate or promote the cleavage.
In addition to their hemostatic activity, platelets also play an active role in inflammation because they contain a plethora of bioactive molecules in their granules [7] and release inflammatory mediators upon activation. Platelets express cytokine receptors that participate in inflammation and can be activated by cytokine bound to these receptors [8]. For example, 14-3-3, which is a GP Ibα-associated adaptor [9], is activated by inflammatory cytokines [10, 11]. Inflammation and thrombosis are therefore considered the tightly interrelated processes that cross-regulate each other [12, 13]. In this regard, understanding the effects of inflammatory cytokines on VWF-bound platelets and their clearance from the vessel wall by ADAMTS13 can provide new insight into the pathophysiology of thrombosis and inflammation and identify new and target therapies.

We examined how IL-6 synergistically interacts with platelets to regulate the clearance of platelet-bearing ULVWF strings under flow conditions. IL-6 is a ubiquitous inflammatory cytokine [14–16] exerting its biological activities through its receptor complex between IL-6 receptor (IL-6Rα) and the co-receptor glycoprotein 130 (gp130) [17, 18]. Platelets express gp130 and can be activated by IL-6 through the trans-signaling mechanism in the presence of soluble IL-6Rα [5, 19–22]. The IL-6 effect is mediated mainly by the Ras/mitogen-activated protein kinase (MAPK), PI3K/Akt, and Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT-3) pathways [5, 23], all of which regulates megakaryocyte differentiation, but are also known to regulate platelet function.

2. Materials And Methods

Ethics statement

Healthy adult volunteers were recruited for blood collection with written informed consent. The study was approved by the Institutional Review Board of Yonsei University, College of Medicine and performed in accordance with the Declaration of Helsinki.

Platelet rich plasma and suspension preparation

To prepare platelet-rich plasma (PRP), we modified the previously published procedures [24]. Briefly, blood samples from healthy donors were drawn into acid citrate dextrose (ACD) tubes. PRP was obtained by centrifugation at 210g for 15 minutes at 22°C. Platelets were pelleted by centrifugation of the PRP at 2,200g for 8 minutes and washed twice with Tyrode’s buffer (10 mM HEPES, 135 mM NaCl, 2.8 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 12 mM NaHCO₃, 0.4 mM NaH₂PO₄, and 5.5 mM glucose; pH 7.4) containing 0.35% human serum albumin (Greencross, Yongin, Korea) and 1 µM prostaglandin I₂ (Sigma Aldrich, St. Louis, MO, USA). Platelets were resuspended in Tyrode’s albumin buffer, counted using an automated hematology analyzer (Coulter counter; ADVIA 2120i, Siemens, Munich, Germany). The platelet concentration was adjusted to meet the purpose of individual experiments.

Fabrication of a microfluidic chip
We fabricated a single straight-channel microfluidic chip using polydimethylsiloxane (PDMS) to monitor ULVWF-mediated platelet binding to the surface of activated endothelial cells. The workloads and time required for procedure were also considerably reduced by replacing flow chambers with microfluidic chips [25]. Briefly, a mold of a microfluidic chip was formed by photolithography, with the microfluidic channel measured 800 µm wide, 125 µm deep, and 28 mm long on a wafer. A 10:1 w/w mixture of PDMS base and curing agent (Sylgard 184, Dow Corning, Midland, MI, USA) was poured over the mold and cured in an oven at 65°C for 2 hours. The solid PDMS was separated from the mold, and two openings were cut at both ends of the microfluidic channel. The PDMS chip was adhered to a glass slide by a corona treater to complete the microfluidic chip and then sterilized in an autoclave.

**Endothelial cell culture in microfluidic chip**

The channel of the microfluidic chip was filled with 1% polyethyleneimine solution and incubated at room temperature for 10 minutes. The chip was washed with distilled water and treated with 0.1% glutaraldehyde for 30 minutes. After washing three times, the chip was coated with 0.3 mg/mL collagen (Sigma) for 20 minutes at 37°C. After coating, the channel was filled with endothelial cell culture medium (EGM-2 bullet kit, Lonza, Walkersville, MD, USA) for 30 minutes at 37°C. At this point, Human umbilical vein endothelial cells (HUVECs, Gibco, Grand Island, NY, USA) culture flasks were detached by trypsin, centrifuged at 180g for 7 minutes, resuspended in cell culture media at a density of 5×10^6 cells/mL and then seeded in the microchip. HUVECs were cultured under static conditions for 24 hrs before experiments.

**Perfusion of platelet suspensions in a microfluidic chip**

Platelets are often used to visualize the ULVWF anchored on endothelial surface in a chamber slide setting. This *in vitro* technique is useful in studying the mechanism of thrombotic disease. HUVECs were grown in the microfluidic chip until full confluence and then activated by 150 µM histamine (Sigma) for 30 minutes at 37°C. The histamine-treated microfluidic chip was placed on an optical microscope stage. Platelet suspensions were treated with 100 ng/mL IL-6 (Sigma) or vehicle for 30 minutes at 37°C, washed once, and then resuspended in Tyrode's albumin buffer. In subsets of samples, platelets were also incubated with 25 µM LY294002 (Sigma), 10 µM U0126 (Cell Signaling Technology, Danvers, MA, USA), or 10 µM R18 (Tocris, Ellisville, MO, USA) for 10 minutes before perfusion. Microtubing connected to the injection pump was linked to the outlet of the microfluidic chip, and a platelet-inhaled microtubing was connected to the inlet. Pretreated platelets were perfused over confluent HUVECs through the chamber with continuous video recording. The flow rate was set at 80 μL/min, which generated 6.4 dynes/cm^2 shear stress with 1 cp viscosity. The number of linear structures in which the ULVWF and the platelets were combined was counted. We also monitored with image recording the removal pattern of platelet-bearing ULVWF strings after perfusing plasma for 4 minutes in a single field of view (original magnification 200×). In our previous study, the metalloprotease activity of ADAMTS13 in plasma was blocked by EDTA, which inhibited string cleavage [25].
Immunoblot
Platelets were treated with 100 ng/mL IL-6 for 30 minutes at 37°C and then lysed with the RIPA buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na$_2$EDTA, 1 mM EGTA, 1% NP40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, and 1 µg/mL leupeptin; Cell Signaling) containing 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) and protease inhibitor cocktail (Roche Applied Science, Mannheim, Germany) for 1 hour on ice. The platelet lysates were centrifuged at 20,000g for 20 minutes at 4°C to remove the insoluble cytoskeletal elements. The supernatants were quantitated using BCA protein assay reagents (Pierce, Rockford, IL, USA), and equal amounts of protein (30-60 µg) were mixed with 5×loading dye. For protein denaturation, mixed protein was heated for 5 minutes at 100°C. Proteins in the total cell lysate were separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis at 70V for 30 minutes and then at 100V for 2 hours. Protein was electrotransferred to 0.45 µm polyvinylidene difluoride membranes (PVDF, GE Healthcare, Munich, Germany) in EzFastBlot transfer buffer (ATTO Corporation, Tokyo, Japan) at 25V for 15 min using a semi-dry transfer unit (ATTO). The membrane was blocked with Tris (tris(hydroxymethyl)aminomethane)–buffered saline-Tween (TBST; 20 mM Tris, 140 mM NaCl, and 0.1% Tween 20) containing 5% bovine serum albumin (BSA) for 1 hour at room temperature and then incubated overnight at 4°C with primary antibodies (dilution between 1:1000 and 1:2000 in TBST with 3% BSA) against 14-3-3 zeta, phospho-14-3-3 zeta, Akt, phospho-Akt, ERK1/2, phospho-ERK1/2, or beta-actin with gentle agitation. After three washes for 5 minutes each with TBST, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000 dilution in TBST with 3% BSA) for 1 hour at room temperature. After additional washing steps, the targeted proteins were visualized with enhanced chemiluminescence (ECL, Pierce) and reagents according to the protocol of the manufacturer using the ImageQuant LAS 4000 mini system (GE Healthcare). We further quantified band intensities with ImageJ software (National Institutes of Health, Bethesda, MD, USA), after normalization to the intensity of beta-actin.

Statistical analysis
Statistical analyses were performed using one-way analysis of variance (ANOVA) and Student’s t-test to evaluate differences between groups. p<0.05 was considered statistically significant.

3. Results And Discussion
Formation of IL-6-treated platelet-ULVWF strings and clearance by ADAMTS13
IL-6, a representative inflammatory mediator, promotes hemostasis through several pathways including platelet aggregation [26, 27]. IL-6-treated platelets are thrombosis-prone in that they are primed for activation by several platelet agonists at subthreshold concentrations [28–30]. We modified the flow-chamber experiments to a miniaturized microfluidic system to perform the experiments with a small volume of blood [25]. We examined IL-6-treated platelets for their influence on the formation of platelet-
bearing ULVWF strings on activated ECs under flow conditions, as compared to mock-treated platelets. As shown in Figure 1A and B, more platelet-bearing ULVWF strings were detected when IL-6-treated platelets were perfused than perfusing untreated platelets (mean ± standard deviation [SD]: 21.6±10.6 versus 16.2±6.5; 1.30±0.16-fold increase; P < 0.01).

(A) HUVECs cultured in a microfluidic channel were stimulated with histamine, and then perfused with platelets untreated (left panel; control) or treated with IL-6 (right panel). ULVWF-platelet formed the beads-on-string appearance (arrow heads) the endothelial surface (original magnification 200x). (B) Data summary from multiple experiments shows fold increase in the number of ULVWF strings, as compared to control (data presented as SEM, n=5, Student’s t test, *P < 0.01).

We further determined whether IL-6-treated platelets influence the cleavage of ULVWF strings, as previously reported [4]. The perfusion of plasma from healthy subjects (as the source of ADAMTS13 shortened the length of preformed ULVWF strings with adherent IL-6-treated platelets or untreated platelets. However, 38.2±16.1% (95% confidence interval [CI] 24.1-52.3; P < 0.01) of ULVWF strings bearing IL-6-treated platelets remained on the endothelial surface after 3 minutes of perfusion, compared with 11.0±5.7% (95% CI 6.0-15.9) of those bearing untreated platelets (Fig. 2).

(A) ULVWF–platelet strings formed during the perfusion of IL-6-treated or untreated platelets were cleaved by ADAMTS13 in plasma and washed away from the endothelial surface (▶ ULVWF-platelet strings initially formed, ▶ Strings showing decrease in length, ▷ Strings eliminated from endothelial surface). (B) Data summary from multiple experiments (Mean±SEM, n=5, *P < 0.05, **P < 0.01 compared to the untreated control).

The platelet IL-6 signaling pathway in clearance of platelet-bearing ULVWF by ADAMTS13

The results demonstrated that IL-6 primed platelets affect the formation and clearance of ULVWF-platelet strings. To explain this phenomenon, we hypothesized that there exists a pathway between the point of the IL-6 signal and VWF-GPIbα binding. It has been shown that the PI3K/Akt and MEK/ERK signal pathways play a role in VWF-GPIb-IX-dependent platelet aggregation [31–33]. Here, we investigated whether IL-6 activates the PI3K/Akt or MEK/ERK pathway in platelets [34, 35] by examining the phosphorylation of Akt or ERK1/2. The IL-6 treatment induced a 1.54-fold (95% CI 1.48-1.60) and 1.63-fold (95% CI 1.25-2.01) increases in the phosphorylation of Akt and ERK, respectively, as compared to the mock treatment (Fig. 3).

Western blot analysis was performed to evaluate Akt and ERK1/2 phosphorylation after the treatment of platelets with 100 ng/mL IL-6 for 30 minutes. β-actin was used as a loading control. Histograms show quantitative analysis of pAkt normalized to total AKT and pERK1/2 normalized to total ERK1/2. Graphs represent the mean±SEM of three separate experiments performed in triplicate, *P < 0.05, **P < 0.01 compared to the untreated group (C).
When platelets treated with IL-6 and the MEK inhibitor U0126 were perfused, the formation of ULVWF strings was reduced by 58.9±9.4% (95% CI 48.2-69.5), as compared to the perfusion of platelets treated with IL-6 alone (Fig. 4). The reduction was 42.9±30.9% (mean±SD, 95% CI 7.9-77.8) with the PI3K inhibitor LY294002, but the difference did not research statistical significance (Fig. 4).

IL-6-treated or resting platelets were treated with or without LY294002 or U0126 and then perfused through activated ECs in the channel. Summarized data presented the related ratio of formed strings. Mean±SEM, n=3, *P < 0.05 compared to the control group, #P < 0.05 compared to the IL-6-treated group.

In contrast, no difference in ULVWF-platelet string formation was observed when untreated platelets were perfused together with either LY294002 or U0126. When plasma was perfused, 84.2±15.2% (95% CI 66.9-100.0) of ULVWF strings bearing untreated platelets were removed within 3 minutes, whereas 52.3±17.5% (95% CI 32.5-72.2) of ULVWF-strings bearing IL-6-treated platelets were eliminated. The reduced cleavage of ULVWF strings bearing IL-6-treated platelet was reversed back to 82.2±16.8% (95% CI 63.3-100.0) by LY294002 (Fig. 5A, 5C). U0126 also increased the cleavage of UVWF strings bearing IL-6-treated platelet from 67.7±7.1% (95% CI 59.7-75.7) to 83.3±5.8% (95% CI 76.8-89.9), comparable to perfusion of mock-treated platelets (81.3±7.0%, 95% CI 73.3-89.3) (Fig. 5B, 5D).

IL-6-treated or untreated platelets were perfused into the channel after treatment with or without LY294002 (A, C) or U0126 (B, D). ULVWF–platelet strings were cleaved by ADAMTS13 contained in plasma and washed away from the endothelial surface. ULVWF-platelet strings initially formed, Strings showing decrease in length, Strings eliminated from the endothelial surface. Summarized data show the clearance rate of ULVWF-platelet strings by ADAMTS13. Mean±SEM, n=3, *P < 0.05 versus the IL-6 only-treated group.

In contrast, inhibitors of JAK or STAT3 did not significant affect the cleavage of ULVWF strings bearing IL-6-treated platelets (data not shown). Together, these results suggest that IL activated PI3K/Akt and MEK/ERK signaling pathways regulated the formation and clearance of VWF-platelet strings formed on activated ECs.

14-3-3ζ regulated the cleavage of ULVWF-platelet strings under flow conditions

14-3-3ζ is a GPIb-IX-associated adaptor protein that interacts with several phosphoserine-dependent binding sites of GPIb-IX [9, 36]. It regulates VWF-GPIIbα binding, VWF-induced GP Ib clustering and mechanosignal transduction leading to platelet activation [9, 37, 38]. Several proinflammatory cytokines were reproted to induce 14-3-3ζ to undergo phosphorylation [10, 11, 36]. We examined whether 14-3-3ζ mediates the effects of IL-6 on ULVWF-platelet string formation and cleavage. Platelets treated with 100 ng/ml IL-6 for 30 minutes had 1.47-fold increase in 14-3-3ζ phosphorylation (95% CI 1.24-1.71) from untreated platelets (Fig. 6).

Western blot analysis was performed to evaluate phosphorylation and protein levels of 14-3-3ζ after treatment of platelets with 100 ng/mL IL-6 for 30 minutes. β-Actin was used as a loading control.
Histograms show quantitative analysis of p14-3-3ζ normalized to β-actin, 14-3-3ζ normalized to β-actin, and p14-3-3ζ normalized to total 14-3-3ζ. Graphs represent the mean±SEM of three separate experiments performed in triplicate, *P< 0.05 compared to the untreated group (C).

When perfusion over activated ECs, the 14-3-3 antagonist R18 did not change the number of ULVWF strings bearing either IL-6-treated or resting platelets (Fig. 7).

IL-6-treated or untreated platelets were perfused into the channel after treatment with or without R18. Many ULVWF-platelet strings were present on the endothelial surface. Summarized data were mean±SEM, n=4, *P < 0.05 compared to control.

However, R18 increased the rate of cleaving ULVWF strings bearing IL-6-treated platelets from 57.4±16.4% (95% CI 41.3-73.4) to 84.9±7.4% (95% CI 77.6-92.1, Fig. 8). These results indicate that 14-3-3ζ regulated the cleavage of ULVWF strings with adherent IL-6-treated platelets. The phosphorylation of 14-3-3 may play an important role in the regulation of protein complex formation and therefore in signal transduction [39, 40].

(A) IL-6-treated or untreated platelets were perfused into the channel after treatment with or without R18. ULVWF-platelet strings were cleaved by ADAMTS13 contained in plasma and washed away from the endothelial surface. ▶ ULVWF-platelet strings initially formed, ▶ Strings showing decrease in length, ▷ Strings eliminated from the endothelial surface. (B) Summarized data showed the clearance rate of ULVWF-platelet strings by ADAMTS13. Mean±SEM, n=4, *P < 0.05 compared to the control group, #P < 0.05 compared to IL-6 alone-treated group.

4. Conclusions

The VWF-platelet complex anchored on the endothelial surface acts as the seed for intravascular thrombus. Its formation and clearance are affected by IL-6, a major inflammatory cytokine, but the exact mechanism is unclear yet. IL-6 primed platelets enhance the ULVWF-platelet complex formation. Also, IL-6 primed platelets are less efficiently cleared by plasma ADAMTS13. PI3K/Akt and MEK/ERK pathways seem to mediate the effect of IL-6 in regulating the formation and the clearance of the ULVWF-platelet complex. IL-6 increases the phosphorylation of 14-3-3ζ, and the inhibition of 14-3-3ζ significantly enhances the clearance of the ULVWF-platelet (IL-6-treated) complex.

Declarations

Declaration of interest

None

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Supplementary data

None

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Figures
Figure 1

Effect of IL-6-induced platelets on the formation of ULVWF strings under flow conditions. HUVECs cultured in a microfluidic channel were stimulated with histamine, and then perfused with platelets untreated (left panel; control) or treated with IL-6 (right panel). ULVWF-platelet formed the beads-on-string appearance (arrow heads) the endothelial surface (original magnification 200x). (B) Data summary from multiple experiments shows fold increase in the number of ULVWF strings, as compared to control (data presented as SEM, n=5, Student’s t test, *P < 0.01).

Figure 2

ULVWF strings with adherent IL-6-treated platelets were partially resistant to cleavage by plasma ADAMTS13 under flow conditions. (A) ULVWF-platelet strings formed during the perfusion of IL-6-treated or untreated platelets were cleaved by ADAMTS13 in plasma and washed away from the endothelial surface (.Strings initially formed, Strings showing decrease in length, Strings eliminated from endothelial surface). (B) Data summary from multiple experiments (Mean±SEM, n=5, *P < 0.05, **P < 0.01 compared to the untreated control).

Figure 3

Activation of the Akt and ERK signaling pathway in IL-6-treated platelets. Western blot analysis was performed to evaluate Akt and ERK1/2 phosphorylation after the treatment of platelets with 100 ng/mL IL-6 for 30 minutes. β-actin was used as a loading control. Histograms show quantitative analysis of pAkt normalized to total AKT and pERK1/2 normalized to total ERK1/2. Graphs represent the mean±SEM of three separate experiments performed in triplicate, *P < 0.05, **P < 0.01 compared to the untreated group (C).

Figure 4

Effect of PI3K and MEK inhibitors on platelet string formation under flow conditions. IL-6-treated or resting platelets were treated with or without LY294002 or U0126 and then perfused through activated ECs in the channel. Summarized data presented the related ratio of formed strings. Mean±SEM, n=3, *P < 0.05 compared to the control group, #P < 0.05 compared to the IL-6-treated group.

Figure 5
The PI3K or MAPK/ERK pathways regulated the cleavage of ULVWF-platelet strings under flow conditions. IL-6-treated or untreated platelets were perfused into the channel after treatment with or without LY294002 (A, C) or U0126 (B, D). ULVWF–platelet strings were cleaved by ADAMTS13 contained in plasma and washed away from the endothelial surface. ▶ ULVWF-platelet strings initially formed, ▶ Strings showing decrease in length, ▷ Strings eliminated from the endothelial surface. Summarized data show the clearance rate of ULVWF-platelet strings by ADAMTS13. Mean±SEM, n=3, *P < 0.05 versus the IL-6 only-treated group.

Figure 6

Phosphorylation of 14-3-3ζ in IL-6-treated platelets. Western blot analysis was performed to evaluate phosphorylation and protein levels of 14-3-3ζ after treatment of platelets with 100 ng/mL IL-6 for 30 minutes. β-Actin was used as a loading control. Histograms show quantitative analysis of p14-3-3ζ normalized to β-actin, 14-3-3ζ normalized to β-actin, and p14-3-3ζ normalized to total 14-3-3ζ. Graphs represent the mean±SEM of three separate experiments performed in triplicate, *P < 0.05 compared to the untreated group (C).

Figure 7

Effect of a 14-3-3 inhibitor on platelet string formation under flow conditions. IL-6-treated or untreated platelets were perfused into the channel after treatment with or without R18. Many ULVWF-platelet strings were present on the endothelial surface. Summarized data were mean±SEM, n=4, *P < 0.05 compared to control.

Figure 8

Involvement of 14-3-3 in the regulation of ULVWF-platelet strings by ADAMTS13 under flow conditions. (A) IL-6-treated or untreated platelets were perfused into the channel after treatment with or without R18. ULVWF–platelet strings were cleaved by ADAMTS13 contained in plasma and washed away from the endothelial surface. ▶ ULVWF-platelet strings initially formed, ▶ Strings showing decrease in length, ▷ Strings eliminated from the endothelial surface. (B) Summarized data showed the clearance rate of ULVWF-platelet strings by ADAMTS13. Mean±SEM, n=4, *P < 0.05 compared to control.
<em>P</em> <lt; 0.05 compared to the control group, <sup>#</sup><em>P</em> <lt; 0.05 compared to IL-6 alone-treated group.</p>