Conformation-Specific Blockade of the Integrin GPIIb/IIIa: A Novel Antiplatelet Strategy That Selectively Targets Activated Platelets

Meike Schwarz, Gerardene Meade, Patrick Stoll, Jari Ylanne, Nicole Bassler, Yung Chih Chen, Christoph E. Hagemeyer, Ingo Ahrens, Niamh Moran, Dermot Kenny, Desmond Fitzgerald, Christoph Bode, Karlheinz Peter

Abstract—Platelet activation causes conformational changes of integrin GPIIb/IIIa (α_{IIb}β_{3}), resulting in the exposure of its ligand-binding pocket. This provides the unique possibility to design agents that specifically block activated platelets only. We used phage display of single-chain antibody (scFv) libraries in combination with several rounds of depletion/selection to obtain human scFvs that bind specifically to the activated conformation of GPIIb/IIIa. Functional evaluation of these scFv clones revealed that fibrinogen binding to human platelets and platelet aggregation can be effectively inhibited by activation-specific scFvs. In contrast to clinically used GPIIb/IIIa blockers, which are all conformation unspecific, activation-specific GPIIb/IIIa blockers do not induce conformational changes in GPIIb/IIIa or outside-in signaling, as evaluated by ligand-induced binding-site (LIBS) exposure in flow cytometry or P-selectin expression in immunofluorescence microscopy, respectively. In contrast to the conformation-unspecific blocker abciximab, activation-specific scFvs permit cell adhesion and spreading on immobilized fibrinogen, which is mediated by nonactivated GPIIb/IIIa. Mutagenesis studies and computer modeling indicate that exclusive binding of activation-specific scFv is mediated by RXD motifs in the heavy-chain complementary-determining region (CDR) 3 of the antibodies, which in comparison with other antibodies forms an exceptionally extended loop. In vivo experiments in a ferric-chloride thrombosis model of the mouse carotid artery demonstrate similar antithrombotic potency of activation-specific scFv, when compared with the conformation-unspecific blockers tirofiban and eptifibatide. However, in contrast to tirofiban and eptifibatide, bleeding times are not prolonged with the activation-specific scFvs, suggesting lower bleeding risks. In conclusion, activation-specific GPIIb/IIIa blockade via human single-chain antibodies represents a promising novel strategy for antiplatelet therapy.

Key Words: thrombosis ■ platelets ■ adhesion molecules ■ GPIIb/IIIa
cells to detect ligand binding to integrin receptors.2,4 GPIIb/IIIa blocker–induced outside-in signaling, although not well understood, may be an inherent property of the currently used strategy for GPIIb/IIIa blockade.3,7,13–16 This property is discussed as a potential explanation for limited efficacy of intravenous GPIIb/IIIa blockers as well as for an increase in mortality seen in several large clinical trials with oral GPIIb/IIIa blockers.3,7,13–16

Another property of the currently used strategy for GPIIb/IIIa inhibition with conformation-unspecific blockers is their inhibitory effects on all circulating platelets with a major risk for bleeding complications. Indeed, bleeding risk assessment is limiting the broader application of GPIIb/IIIa blockers in various groups of patients such as the elderly and patients treated with fibrinolytics.17 GPIIb/IIIa blockers that bind to the receptor solely in its activated state could circumvent many of the problems described above. Therefore, we designed a strategy to generate activation-specific GPIIb/IIIa blockers. We selectively synthesized single-chain antibodies (scFvs), which are a minimal form of functional antibodies, consisting of the variable regions of only the heavy and light chain of the antibody, connected by a small linker peptide. We constructed scFv phage libraries and developed a novel subtractive panning protocol, based on the sequential use of the native and activated integrin receptor.18 Thereby, we were able to select a panel of scFvs that bind specifically to activated GPIIb/IIIa.19 In the present study, we evaluate the concept of activation-specific blockade of platelet integrin GPIIb/IIIa via these novel human single-chain antibodies in direct comparison with the conformation-unspecific blockade of GPIIb/IIIa.

Methods and Materials

Generation of the Single-Chain Antibodies MA2 and SCE5

The generation of 2 scFv phage libraries and the selection procedure of activation-specific scFvs has been recently described in detail.17 For 1 library, the natural library, PCR was used to amplify the variable region of the heavy and light chain from cDNA, which was prepared using peripheral blood lymphocytes from healthy donors and spleen biopsies. For the second library, the synthetic library, the CDR3 region of the heavy chain was randomized in scFv formats, for which a high bacterial expression has been demonstrated. Single-chain antibodies were produced as described in detail.18 Briefly, scFv were cloned in the expression vector pHOG-21 and transformed into Escherichia coli TG-1. Bacteria were induced with 0.4 mol/L sucrose and 0.25 mmol/L isopropyl-β-D-galactoside (Sigma) and incubated for 16 hours at 200 rpm and 23°C. The bacteria were lysed with an ice-cold hypotermic shock solution (20% sucrose, EDTA, Tris), and scFvs were purified by a 2-step FPLC-procedure, using metal-affinity chromatography followed by anion-exchange chromatography.

Flow Cytometry

Whole blood was diluted 1/50 in Tyrode’s buffer (150 mmol/L NaCl, 2.5 mmol/L KCl, 12 mmol/L NaHCO3, 2 mmol/L MgCl2, 2 mmol/L CaCl2, 1 mg/mL BSA, 1 mg/mL dextrose; pH 7.4), partially activated through addition of 20 μmol/L ADP, 100 ng/mL phorbol 12-myristate 13-acetate (PMA), or 50 μg/mL collagen-related peptide (CRP) (gift of Dr Jackson, Melbourne, Australia), and then preincubated for 10 minutes with purified scFv, abciximab (Eli Lilly), epitifibatide (Millisenum), GRGDSP peptide (Biomol), or no addition. Secondary staining was performed with Alexa-Fluor-488–labeled monoclonal anti–His tag antibody (Qiagen), a dichlorotriazineylaminofluorescein (DTAF)–labeled polyclonal goat anti-mouse IgG+IgM (H+L) antibody (for abciximab staining; Jackson), a polyclonal fluorescein isothiocyanate (FITC)–labeled rabbit anti-human fibrinogen antibody (Dako), or a FITC–labeled anti–LIBS-145 antibody.20 After fixation with CellFIX (Becton-Dickinson), samples were measured in by fluorescence-activated cell sorting (FACS-Calibur, Becton-Dickinson).

Alignment and Computed Modeling

Alignment of the amino acid sequence of the heavy-chain CDR3 region of scFv MA2 in comparison to the murine antibodies PAC-1 (GenBank accession no. J04439) and OPG-2 (NRL3D: 1BM3H) was performed using the Clustal multiple-alignment program (German Cancer Research Institute). The MA2 scFv was modeled based on structures 1hdq19 and ladql20, which showed 81.2% and 99.5% sequence identity to the heavy- and light-chain regions of MA2, respectively. For domain orientation, the scFv structure 1F3c21 was used. The linker region between the heavy and light chains was not included in the model. Superimposition was done with the program O and minimization of the loops with Insight-II software (Accelrys). Finally, the structure was visualized using a Rasmol-pdb viewer (University of Massachusetts).

Immunoprecipitation

We performed immunoprecipitations of resting and ADP-stimulated (20 μmol/L) platelets as described by Longhurst et al.22 MA2 was incubated with platelets at 10 μg/mL. Complexes were captured with NiNTA-agarose (Qiagen) and eluted with IMAC-elution buffer (50 mmol/L NaH2PO4, 300 mmol/L NaCl, 250 mmol/L imidazole, pH 8). After SDS-gel-electrophoresis and Western blotting, the membrane was blocked with 1% BSA and hybridized either with streptavidin–horseradish peroxidase (HRP) to detect total surface proteins or with a mouse anti-CD41 mAb (Immunotech) or a polyclonal goat anti-CD61 antibody (Santa Cruz Biotechnology) to detect the GPIIb/IIIa subunits. Secondary hybridization was performed with polyclonal HRP-labeled secondary antibodies (Santa Cruz Biotechnology).

Immunofluorescence Staining of Platelets Adhering to Collagen

Glass cover slips were incubated with collagen (50 μg/mL, Horm Type-I, Nycomed) at 4°C over night and blocked with 1% BSA. Washed platelets were prepared from citrated platelet-rich plasma (PRP) using a Sepharose–CL-2B column (Sigma). After elution, platelets were incubated with ADP, MA2, abciximab, or no addition for 15 minutes at room temperature. Then, platelets were allowed to adhere on the coated cover slips for 30 minutes at 37°C, stained with an anti-CD62P antibody, a FITC–labeled Fc-specific goat anti-mouse antibody (Jackson), and fixed. Photographs were taken on a Zeiss-Axioplan-2 microscope.

Aggregometry

Aggregometry was performed in a Biodata PAP-4 aggregometer. After incubation with the scFv for 10 minutes at 37°C, aggregation was induced by addition of 20 μmol/L ADP with stirring at 900 rpm. The titration curves were evaluated with Prism 3 software (Graph-
Adhesion Assay

Glass cover slips were coated over night with purified fibrinogen (Calbiochem, 30 μg/mL). CHO cells were washed with PBS and incubated with MA2, abciximab (both 10 μg/mL), or no addition for 15 minutes. Cells were allowed to adhere for 30 minutes at 37°C. Cover slips were washed 3 times in PBS, and photographs were taken using a Zeiss-Axioplan-2 microscope.

GPIIb/IIIa-Mediated Platelet Adhesion and Aggregation Under Flow

Cover slips coated with fibrinogen (100 μg/mL) were placed in a modified parallel plate flow chamber (GlycoTech). Untreated PRP, adjusted to 280 to 300×10^4/μL, was used as a control. PRP was incubated with abciximab (10 μg/mL) or with MA2 (50 μg/mL) for 10 minutes. Platelets were then perfused through the flow chamber at a shear rate of 50 sec^-1 using a syringe pump (Harvard Apparatus) at 37°C. Phase contrast microscopy images were taken on a Zeiss-Axiovert-200-epifluorescence microscope.

Platelet Function Analyzer Assay

The platelet function analyzer assay (PFA-100; Dade Behring) was used to evaluate the effect of increasing concentrations of either abciximab or scFv MA2 in human whole blood. Citrated blood was incubated with antibodies for 30 minutes at room temperature. Closure time was measured using cartridges containing a membrane either coated with epinephrine/collagen or ADP/collagen.

In Vivo Functional Evaluation of Antithrombotic Efficacy and Protection From Bleeding in a Mouse Model

C57BL/6 mice (22 to 38 g, Charles River) were used. Care and use of laboratory animals followed the national guidelines and was approved by the institutional animal care and ethics committees. An incision of the skin was made in anesthetized mice directly on the top of the right common carotid artery region. The fascia was bluntly dissected and a segment of the right common carotid artery was excised. A nano-Doppler flow probe (0.5 VB, Transonic) was exposed. A segment of the right common carotid artery was dissected and a segment of the right common carotid artery was exposed. A nano-Doppler flow probe (0.5 VB, Transonic) was positioned over the artery and the carotid blood flow was measured by a flowmeter (T106, Transonic). Thrombosis was induced by applying a filter paper (1×2 mm) saturated with ferric chloride (10%) under the right carotid artery for 3 minutes. Thrombotic occlusion was defined by a drop in flow to <0.2 mL/min. One minute before the ferric chloride treatment, mice were infused through the tail vein with saline, tirofiban, eptifibatide, and scFv MA2 (10 μg/mL) for 10 minutes. Platelets were then perfused through the flow chamber at a shear rate of 50 sec^-1 using a syringe pump (Harvard Apparatus) at 37°C. Phase contrast microscopy images were taken on a Zeiss-Axiovert-200-epifluorescence microscope.

Results

To obtain human single-chain antibodies that bind exclusively to the activated conformation of GPIIb/IIIa, we constructed scFv phage libraries with high complexities of up to 10^9 independent clones. We developed a new subtractive selection procedure with depletion for nonactivated GPIIb/IIIa and selection for activated GPIIb/IIIa. The differing conformations of GPIIb/IIIa were provided either on nonactivated or activated platelets or on CHO cells expressing the native or a mutated and, therefore, constitutively activated form of GPIIb/IIIa. Using this unique selection procedure, we successfully selected over 20 activation-specific anti-GPIIb/IIIa scFv clones. The scFv clone demonstrating strongest binding to human platelets, MA2, binds exclusively to activated platelets, in contrast to the Fab fragment abciximab, which also binds to nonactivated platelets (Figure 1A). In addition to MA2, the scFv SCE5, which cross-reacts with mouse platelets, as described later, was evaluated (Figure 1A). Specificity for GPIIb/IIIa as well as for its activated, high-affinity form could also be proven by flow cytometry using CHO cells expressing recombinant GPIIb/IIIa in its native and activated form (data not shown). Furthermore, the activation specificity of MA2 could also be demonstrated by immunoprecipitation. On activated platelets, MA2 precipitated 2 surface proteins of approximately 100 and 125 kDa, which were identified by immunoblotting with antibodies specific for the 2 subunits of GPIIb/IIIa (Figure 1B). In contrast, there were no bands detected when nonactivated platelets were used for immunoprecipitation.

The clinically used GPIIb/IIIa blockers bind to nonactivated GPIIb/IIIa and induce a conformational change of GPIIb/IIIa that results in the exposure of LIBS epitopes. Using the anti–LIBS-145 mAb,12 we show that activation-specific scFvs, in contrast to GRDSP peptide and the GPIIb/IIIa blocker abciximab as well as eptifibatide, do not provoke LIBS expression (Figure 2A). The conformational change of GPIIb/IIIa induced by the binding of non–conformation-specific GPIIb/IIIa blockers may result in outside-in signaling, as generally seen with ligand binding to integrins. Indeed, when platelets adhering to a collagen matrix are incubated with abciximab, outside-in signaling can be de-
eptifibatide (10 μg/mL) or no stimulation. Reproduced to represent an “in vitro bleeding time” assay.25 A clear contrast, MA2 does not prolong closure time with ADP (0.5 mmol/L), abciximab (10 μg/mL), or no stimulation. Representative photographs of 5 experiments are shown.

**Figure 2.** Induction of conformational changes and outside-in signaling of GPIIb/IIIa by non–conformation-specific but not by activation-specific GPIIb/IIIa blockers as detected by LIBS exposure and P-selectin expression, respectively. A, MA2 and SCE5 (both 10 μg/mL) do not induce LIBS epitope expression on nonstimulated platelets, in contrast to abciximab (10 μg/mL), epifibatide (10 μg/mL), and the GRGDSP peptide (2 mmol/L). Depicted is the mean fluorescence of FITC-labeled anti–LIBS-145 mAb (10 μg/mL), as detected by flow cytometry in triplicate experiments. B, Immunofluorescence microscopy of P-selectin expression as a measure of outside-in signaling on the surface of adhering platelets after incubation with ADP (20 μmol/L), MA2 (10 μg/mL), abciximab (10 μg/mL), or no stimulation. Representative photographs of 5 experiments are shown.

**Figure 3A.** DNA sequencing of MA2 revealed that it contains an RND loop region resulted in a loss of activation specificity (Figure 3C). To evaluate further the GPIIb/IIIa-binding determinants of MA2, we developed a molecular model based on published 3D structures of scFvs and human heavy and light-chain segments (Figure 3D). This model suggests that the HCDR3 loop of MA2 is remarkably long and protruding.

Cross-reactivity with integrin α,β3 was evaluated in flow cytometry using CHO cells expressing α,β3. Neither binding to native α,β3 nor to α,β3 activated by 0.5 mmol/L Mn2+ could be detected for MA2 (data not shown), indicating selectivity for GPIIb/IIIa.

To evaluate whether activation-specific scFvs are capable of inhibiting fibrinogen binding in a manner comparable to conformation-unspecific GPIIb/IIIa blockers, we tested various strong platelet stimuli including CRP and PMA. For both stimuli, we could demonstrate similar inhibitory potency for activation-specific scFvs and abciximab (Figure 4A). As the standard test for the functional evaluation of platelets, we also performed light-transmission aggregometry. Both abciximab and activation-specific scFvs were able to block completely ADP-induced platelet aggregation (Figure 4B).

In contrast to soluble fibrinogen, immobilized fibrinogen is capable of binding to both activated and nonactivated GPIIIa. This mechanism is of physiological importance, as it allows the adherence of nonactivated platelets to immobilized fibrinogen on the vessel wall at sites of vascular injury.24 We used CHO cells expressing native and activated GPIIb/IIIa as a model to investigate distinct antiadhesive properties of GPIIb/IIIa blockers. Whereas abciximab inhibited the adhesion of GPIIb/IIIa-expressing CHO cells irrespective of the activation status of GPIIb/IIIa, MA2 only inhibited the adhesion of cells expressing activated GPIIb/IIIa (Figure 5).

To confirm the specificity of MA2 for activated GPIIb/IIIa in an in vivo correlate, we investigated its effect, in comparison with abciximab, on GPIIb/IIIa-mediated platelet adhesion to immobilized fibrinogen under flow conditions. As depicted in Figure 6, although both blockers completely abrogated platelet aggregation under flow, GPIIb/IIIa-mediated platelet adhesion and spreading to immobilized fibrinogen was still possible, even under high concentrations of MA2.

To evaluate the effects of activation-specific scFvs on human hemostasis, we used the PFA-100, which is considered to represent an “in vitro bleeding time” assay.26 A clear difference in closure time between the non–conformation-specific abciximab and the activation-specific scFv MA2 could be seen (Figure 7). Abciximab prolongs closure time at concentrations (2.5 μg/mL) achieved in clinical practice. In clear contrast, MA2 does not prolong closure time with ADP stimulation and prolongs closure time only slightly with epinephrine stimulation at high concentration (10 μg/mL). This difference between ADP and epinephrine is in accordance with the higher sensitivity for platelet inhibition by the weaker agonist epinephrine reported by the manufacturer. For the scFv SCE5, similar results were obtained as for scFv MA2 (data not shown). Overall, the results obtained with PFA-100 suggest that the bleeding tendency in humans may be reduced with activation-specific in comparison with non–conformation-specific GPIIb/IIIa blockers.
To demonstrate the advantages of activation-specific GPIIb/IIIa blockade in vivo, we chose a ferric chloride–induced mouse carotid artery thrombosis model. ScFv MA2, with an optimal binding to human platelets, did not bind to mouse platelets. However, 1 of the clones obtained from the synthetic library, SCE5, demonstrated strong binding to mouse platelets (data not shown). In direct comparisons with MA2, SCE5 demonstrated nearly similar properties of activation-specific binding and blockade of GPIIb/IIIa (Figures 1, 2A, and 4). SCE5 was compared with the currently clinically used GPIIb/IIIa blockers eptifibatide and tirofiban (abciximab, similar to MA2, did not bind to mouse platelets). The antithrombotic effect of SCE5 was comparable to eptifibatide and tirofiban (Figure 8A). However, in contrast to eptifibatide and tirofiban, the scFv did not prolong bleeding times (Figure 8B and 8C). The often-used tail transection (Figure 8B) as well as an incision method (Figure 8C), which resembles the template bleeding times determined in humans, revealed normal bleeding times. Moreover, the combination of an anticoagulant (enoxaparine) and a GPIIb/IIIa blocker, which is a typical combination used in the clinic, revealed an even stronger difference between eptifibatide and the activation-specific scFv (Figure 8C). Thus, our data suggest a major reduction of bleeding risk for activation-specific GPIIb/IIIa blockade.

**Discussion**

The adhesion-molecule group of integrins share the property to change conformation, on cell activation, thereby exposing their receptor ligand—binding pocket. This provides the unique possibility to design agents that specifically block only the activated receptor. We designed a strategy to develop human single-chain antibodies specific for integrins in the activated conformation that could be used for the therapy of the many diseases in which integrins play a major role, such as inflammation, cancer, and thrombosis. The platelet integrin GPIIb/IIIa is of pivotal importance for coagulation and thrombosis. Its conformation-unspecific blockade has been 1 of the major advances in antithrombotic therapy of recent years, although unexpected limitations and side effects, in particular with orally applicable blockers, have damped the original enthusiasm. We have taken advantage of the existence of different conformational states of GPIIb/IIIa to develop an alternative, unique strategy that targets only activated platelets. The versatile technology of single-chain antibody phage display, which permits an in vitro imitation of the natural immunization process, in combination with a unique subtractive panning strategy, provides the capability to select antibodies against discretely differing epitopes. This strategy is unique in its property to target complex cell membrane proteins, such as integrins, which cannot be purified and/or immobilized without loss of integrity and/or function-determining conformation.

Based on our data, we see several major advantages of an activation-specific GPIIb/IIIa blockade. (1) Because activation-specific scFvs do not induce exposure of LIBS epitopes and because they are of complete human and thereby probably nonimmunogenic origin, the induction of thrombocytopenia is unlikely. 2) Outside-in signaling, which is discussed as an inherent property of non–conformation-
specific, ligand-mimetic GPIIb/IIIa blockers, was not seen with the activation-specific scFvs. The relevance of this difference for the clinical use of GPIIb/IIIa blockers remains to be determined. 3) The obtained in vitro and in vivo data promise a reduction in bleeding complications. The recently reported finding that the initial thrombus (plug) formation after vessel injury can be mediated by nonactivated platelets, whereas the further development of a larger and stable thrombus is dependent on platelet activation, provides a mechanistic model for the advantageous mode of action of activation-specific GPIIb/IIIa blockade: activation-specific scFvs do not inhibit adhesion of nonactivated platelets via their nonactivated GPIIb/IIIa and thus do not block the initial platelet plug. In contrast, activation-specific scFvs block activated platelets via their activated GPIIb/IIIa receptor and thus inhibit thrombus growth. In addition as shown in our experiments, activation-specific scFvs do not inhibit platelet adhesion to immobilized fibrinogen, which is present on injured vessel walls, and thus, the formation of an initial platelet monolayer at the site of vascular injury via adhesion.

Figure 4. Inhibitory effects of activation-specific scFvs. A, Inhibition of fibrinogen binding in flow cytometry. The histograms demonstrate binding of fibrinogen either to platelets activated by ADP (20 μmol/L), CRP (50 μg/mL) or PMA (100 ng/mL) or to nonactivated platelets (no addition). Fibrinogen binding to activated platelets is inhibited by MA2, SCE5, or abciximab (10 μg/mL each), independent of the platelet agonist used. B, Light-transmission aggregometry. Inhibition of platelet aggregation, induced by 20 μmol/L ADP, is monitored at various concentrations of MA2, SCE5, and abciximab.
of nonactivated platelets to fibrinogen is possible. These mechanistic models may explain the differential effects of activation-specific GPIIb/IIIa blockade on the development of large thrombi in the arterial system compared with bleeding on vessel injury.

Additional advantages are provided by the scFv format, which indeed is currently entering the phase of clinical trials.27,28 The ease of cloning in bacteria offers the opportunity of genetic engineering, such as further optimization and the construction of fusion molecules.29 Activation-specific single-chain antibodies can be used for clot targeting of anticoagulants and/or fibrinolytics. Thus, highly selective agents specifically inhibiting only activated platelets and clot-targeted anticoagulants and/or fibrinolytics can be generated in a single unique molecule.30 Besides the therapeutic options, the antibodies described provide interesting perspectives as diagnostic markers and imaging tools. Preliminary results demonstrate that activation-specific scFvs can be used to diagnose platelet activation in clinical settings, such as acute coronary syndrome, and to detect thrombi and emboli in magnetic resonance imaging (unpublished data). In contrast to the production of antibodies in hybridoma or other mammalian cells, scFvs can be produced in bacteria at low cost and are easily amenable to commercial scale up. Another technical advantage is the possibility to add functional groups (eg, His tag) to allow for high-purity production using affinity columns.

Recently published structures investigating the complex binding between the extracellular segment of integrin GPIIb/IIIa and ligand-mimetic GPIIb/IIIa blockers show that the aspartic acid (D) side chain coordinates with a metal in the MIDAS (metal ion–dependent adhesion site) region of the H9252 subunit, whereas the arginine (R) side chain interacts with the H9252 -propeller domain of the IIb subunit.31 According to these observations, specificity for the activated GPIIb/IIIa could be obtained by constraining the conformations of the aspartic acid and arginine side chains or by yet undefined interactions. The length and the protruding conformation of the HCDR3 loop define unique features of the activation-specific scFv. The sequence and structural information, in particular of the HCDR3 region, acquired in this study provide a basis for the generation of a structural model of an activation-specific GPIIb/IIIa blocker, providing a template for the development of activation-specific low–molecular mass blockers that ultimately may be administered orally.

In summary, we generated activation-specific single-chain antibodies that bind exclusively to the activated GPIIb/IIIa. We provide evidence that the scFv phage-display technology, in combination with a newly developed subtractive panning
protocol, can be used to design agents that allow a conformation-specific blockade of highly complex cell membrane proteins. The GPIIb/IIIa-blocking potency of these scFvs is comparable to the clinically used GPIIb/IIIa blockers, such as abciximab. However, in contrast to abciximab, activation-specific scFvs do not cause ligand-induced conformational changes of GPIIb/IIIa and do not block resting platelets. In vivo evaluation revealed an antithrombotic effect that is comparable to the clinically used GPIIb/IIIa blockers tirofiban and eptifibatide. Most notably, activation-specific GPIIb/IIIa blockade via scFv did not prolong bleeding times, in clear contrast to the conformation-unspecific GPIIb/IIIa blockers tirofiban and eptifibatide. Overall, the conformational change of integrin GPIIb/IIIa on platelet activation provides the basis for the design of a unique and promising therapeutic strategy that selectively targets activated platelets.

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**Disclosures**
None.

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