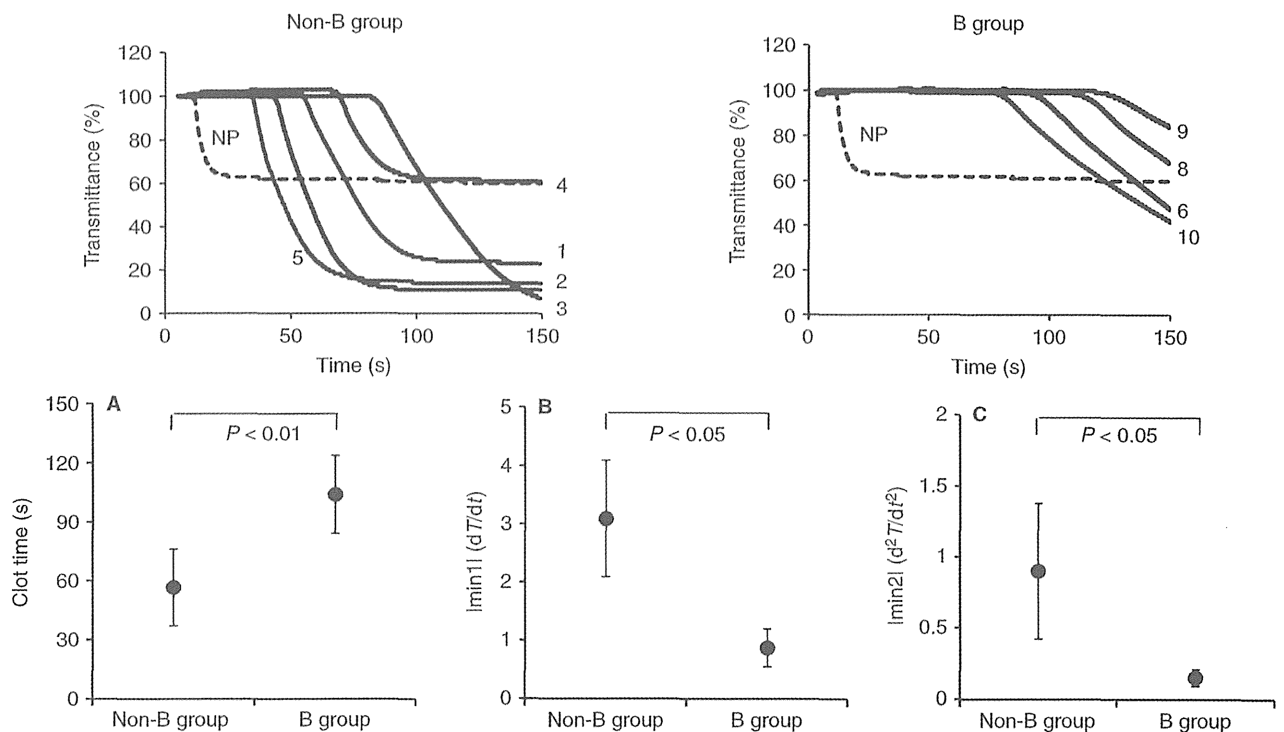

Fig. 1. Clot waveform analysis in plasmas from patients with acquired factor V inhibitors. The prothrombin times in patients' plasmas in both groups were measured with the MDA-II system. The parameters (lower panels) clot time (A), min1 (B) and min2 (C) were calculated from the clot waveform (upper panels) observed, as described in Materials and methods. In all instances, results are shown as mean ± standard deviation from at least five separate experiments. NP and lines 1–10 refer to normal plasma and cases 1–10. Because of the small volume of the sample from case 7, this case failed to show the curve of clot waveform.

the B group inhibited this binding dose-dependently (by 60–90%; IC_{50}, 10–30 μg mL^{-1}), whereas those from the non-B group did not significantly inhibit binding (i.e. by < 15%), even at the maximum concentration employed (Fig. 3). These findings strongly suggested that the severe hemorrhagic tendency in the B group was associated with significant inhibition of FV binding to PL by the anti-FV inhibitors.

Effects of anti-FV autoAbs on prothrombinase activity

The effects of anti-FV autoAbs on the activity of the prothrombinase complex were examined in a purified assay, even though thrombin generation appeared to be equally depressed in all patients. All of the available IgGs from the B group significantly inhibited prothrombinase activity dose-dependently, by > 90% at the maximum concentration, probably because of failure of FV(a)–PL binding. Similarly, all IgG preparations from the non-B group also depressed prothrombinase activity dose-dependently, but this inhibition (by 50–60%) at the maximum concentration was comparatively modest (Fig. 4). The autoAbs from the non-B group had little effect on FV(a)–PL binding, and it might be that inhibition of prothrombinase activity in these circumstance reflected interactions of FVa with other components of the complex (FXa and/or prothrombin).

APC sensitivity in the presence of A-FV inhibitors

An alternative function of FV is as an anticoagulant cofactor of APC in FVIIIa inactivation [6], and it seemed possible that the asymptomatic phenotype in some patients with FV autoAbs might be attributable to some effect of the acquired inhibitor on these secondary properties of FV. To investigate this, APC-mediated inactivation of FVIIIa with anti-FV inhibitor plasmas was examined in an intrinsic FXa generation assay. FXa generation was determined with a plasma-based assay with exogenous APC. Figure 5A shows absorbance readings in this assay in the absence or presence of exogenous APC, and Fig. 5B shows the calculated APCsr. The results indicated that the APCsr in the B group (2.05 ± 0.08) was lower than that in normal plasma (2.45 ± 0.03), but was markedly decreased in the non-B group (1.45 ± 0.13). The APCsr in the non-B group appeared to be similar to that observed with APCR plasmas with FV Leiden or FV Nara (~ 1.5) [27,28].

Effects of anti-FV autoAbs on the APC cofactor activity of FV

The APC cofactor function of FV in FVIIIa inactivation was further examined in a purified assay. The initial rates

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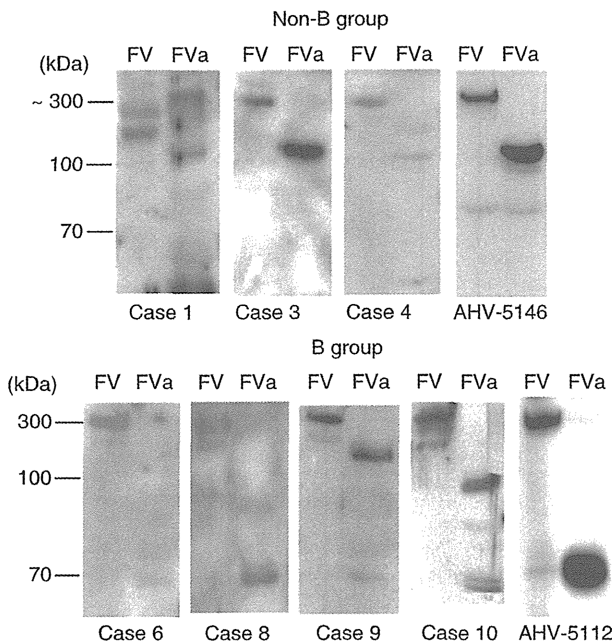

Fig. 2. Identification of epitope(s) of acquired factor V inhibitors. Samples of FV and thrombin-cleaved FVa (100 nM) were analyzed by 8% gel SDS-PAGE, and this was followed by transfer to poly (vinylidene difluoride) membranes. The membranes were incubated with patients' plasmas, and binding to FV(a) fragments was detected by further incubation with anti-human peroxidase-linked secondary antibody. As a positive control, detection with AHV-5146 (heavy chain) and AHV-5112 (light chain) are shown.

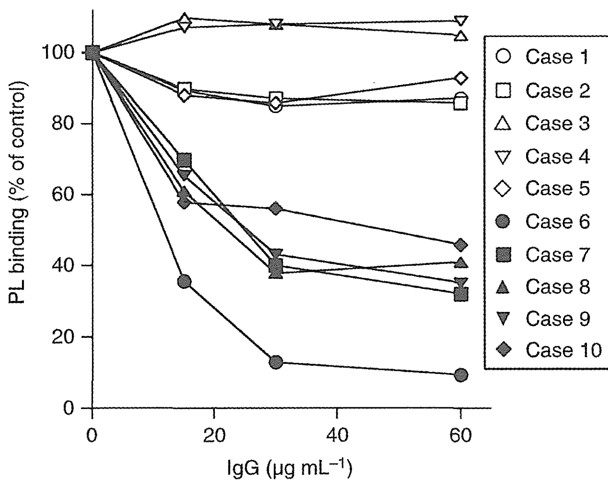

Fig. 3. Effects of anti-FV autoantibodies (autoAbs) on FV binding to phospholipid (PL) in solid-phase-based ELISA. α-Phosphatidyl-L-serine (5 μg mL^{-1}) in methanol was added to microtiter wells, and air-dried at 4 °C overnight. After blocking with gelatin solution, the reactant mixtures with FV (1 nM) and various concentrations of anti-FV autoAbs were added to the PL-coated well. Bound FV was quantified with anti-FV mAb as described in Materials and methods. The absorbance of FV binding to PL without anti-FV autoAb represents 100%. Binding to FV in the presence of normal IgG was used as a control. The percentage of FV binding was plotted as a function of the anti-FV autoAb concentration. All experiments were performed at least three separate times, and the average values are shown.

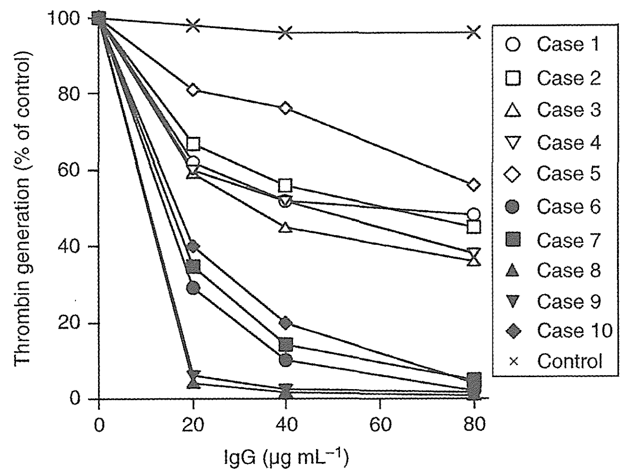

Fig. 4. Prothrombinase activity in the presence of anti-FV autoantibodies (autoAbs). Mixtures of FVa (2 nM) and anti-FV autoAbs were added to prothrombin (1.4 μM), phospholipid vesicles (20 μM), and 5-dimethylamino-naphthalene-1-sulfonylarginine-*N*-(3-ethyl-1,5-pentanediyl)-amide (30 μM). Thrombin generation was initiated by the addition of FXa (10 pM) as described in Materials and methods. Rates of thrombin generation were determined at 405 nm, after the addition of S-2238. The initial rate of thrombin generation without anti-FV autoAb represents 100%. The percentage of prothrombinase activity was plotted as a function of the anti-FV autoAb concentration. All experiments were performed at least three separate times, and the average values are shown.

of FXa generation are shown in Fig. 6. Control experiments demonstrated that FXa generation in the absence of APC (~ 205 nM) was reduced by the presence of APC (~ 175 nM), and was further reduced when both APC and FV were present (~ 145 nM), again confirming FV cofactor activity in the APC-mediated inactivation of FVIIIa. Mixtures of FV with anti-FV autoAbs from all patients in the non-B group enhanced FXa generation dose-dependently, whereas the results obtained with similar mixtures containing anti-FV autoAbs from the B group were not significantly different from those obtained with normal IgG. These results strongly indicated that anti-FV autoAbs from the non-B group impaired the APC cofactor activity of FV, and inhibitors from the B group had little effect on this function of FV.

Effects of anti-FV autoAbs on APC-catalyzed cleavage of FVa HCh

FV/FVa-related APCR is governed by reduced sensitivity of FVa to APC-mediated inactivation and/or impairment of the APC cofactor activity of FV in FVIIIa inactivation. Experiments were therefore designed to investigate the effects of anti-FV autoAbs on APC-mediated proteolytic inactivation of FVa (Fig. 7). The time-related cleavage of HCh was analyzed by SDS-PAGE/western blotting with an anti-FV HCh mAb recognizing residues 307–506. When FVa and normal IgG were used, the band representing residues 1–506 rapidly appeared within

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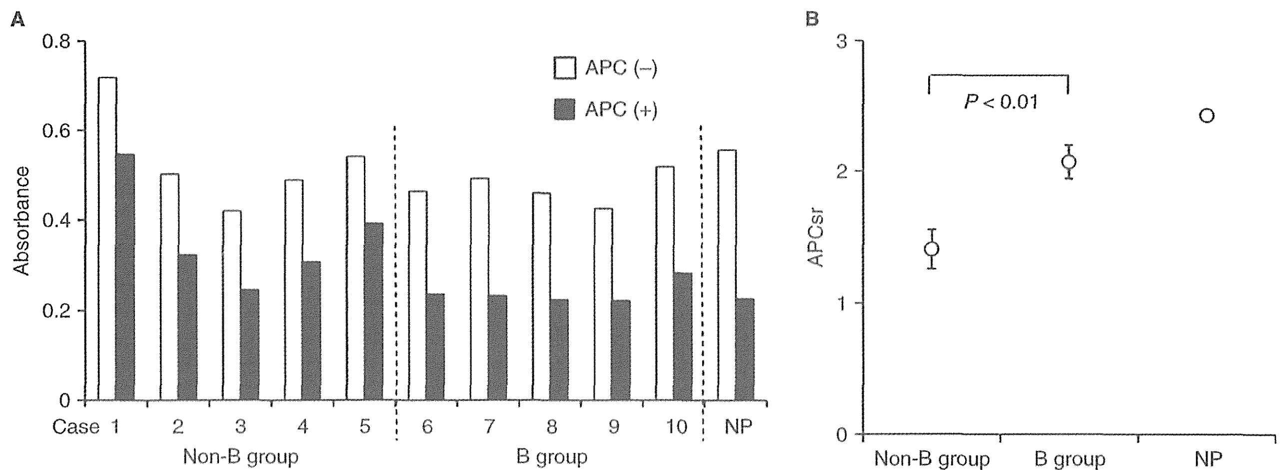

Fig. 5. Activated protein C (APC) sensitivity ratio (APCsr) in the non-B group or B group with acquired factor V inhibitors. FXa generation assays in the presence of APC with patients' plasmas or normal plasma (NP) were performed with COATEST SP FVIII as described in Materials and methods. (A) The absorbance readings for FXa generation obtained in the absence (open bars) or presence (solid bars) of exogenous APC (40 nM) in both groups. The APCsr shown in (B) was expressed as the absorbance obtained in the absence of APC divided by that in its presence. Results are shown as mean ± standard deviation from at least five separate experiments.

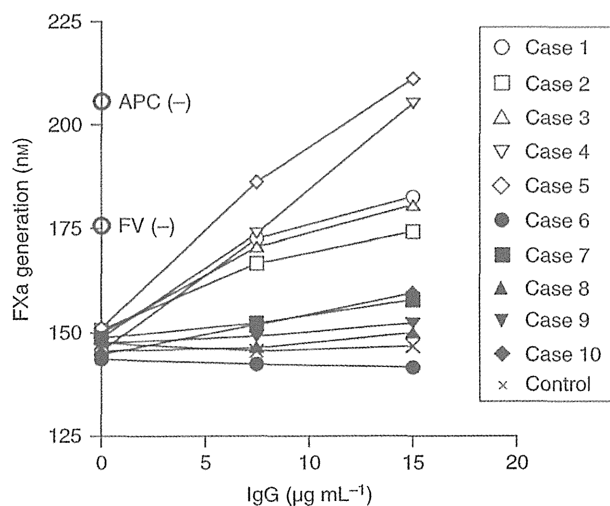

Fig. 6. Effects of anti-FV autoantibodies (autoAbs) on the activated protein C (APC) cofactor activity of FV assessed by FVIIIa degradation. FVIII (10 nM) and phospholipid (20 μM) were activated by thrombin (5 nM), and this was followed by the addition of hirudin. Generated FVIIIa was incubated with APC (1 nM) and protein S (4 nM) together with mixtures of FV (1 nM) and anti-FV inhibitor autoAbs (7.5 and 15 μg mL^{-1}) for 20 min. FXa generation was initiated by the addition of FIXa (2 nM) and FX (200 nM) for 1 min, and the generated FXa was measured by the addition of S-2222. The initial rates of FXa generation were expressed as the FXa generation calculated from the standard reference curve for FXa. Control experiments were performed in the absence of either APC or FV. All experiments were performed at least three separate times, and the average values are shown.

2 min after the addition of APC, and this was followed sequentially by strong band representing residues 307–506, and a faint band representing residues 307–709. These patterns were consistent with rapid, consecutive cleavage of FVa at Arg506 and Arg306. The cleavage patterns of FVa with anti-FV autoAbs in the non-B group demonstrated the appearance of bands representing residues 1–506 from all cases, except for case 5, at a similar velocity to that obtained with control IgG, but the appearance of bands representing residues 307–506 from all cases appeared to be markedly delayed. In all instances, densitometry analysis also demonstrated a 50–90% reduction relative to control in the intensity of bands representing residues 307–506, suggesting inhibition of cleavage at Arg306 but not at Arg506. In all B group cases, however, APC cleavage of HCh was significantly inhibited or completely abolished at both Arg306 and Arg506, owing to the inhibition of FVa–PL binding by anti-FV autoAbs (data not shown). Taken together, these findings suggest that the APCR in the non-B patients could be attributable not only to impairment of the APC cofactor activity of FV in FVIIIa inactivation, but also to reduced APC-mediated inactivation of FVa, related to significant delay in cleavage at Arg306.

Discussion

We investigated coagulation activity and functional mechanisms, associated with either asymptomatic or hemorrhagic symptoms, in patients with A-FV inhibitors. The results indicated that the clinical phenotype in these patients was dependent on specific epitopes of the anti-FV autoAbs. Our evidence is based on the following findings: (i) the PT-based CWA showed lower hemostatic function in the B group than in the non-B group; (ii) anti-FV autoAbs from the B group reacted predominantly with the LCh, whereas those from the non-B group reacted more with the HCh; (iii) anti-FV autoAbs from the B group

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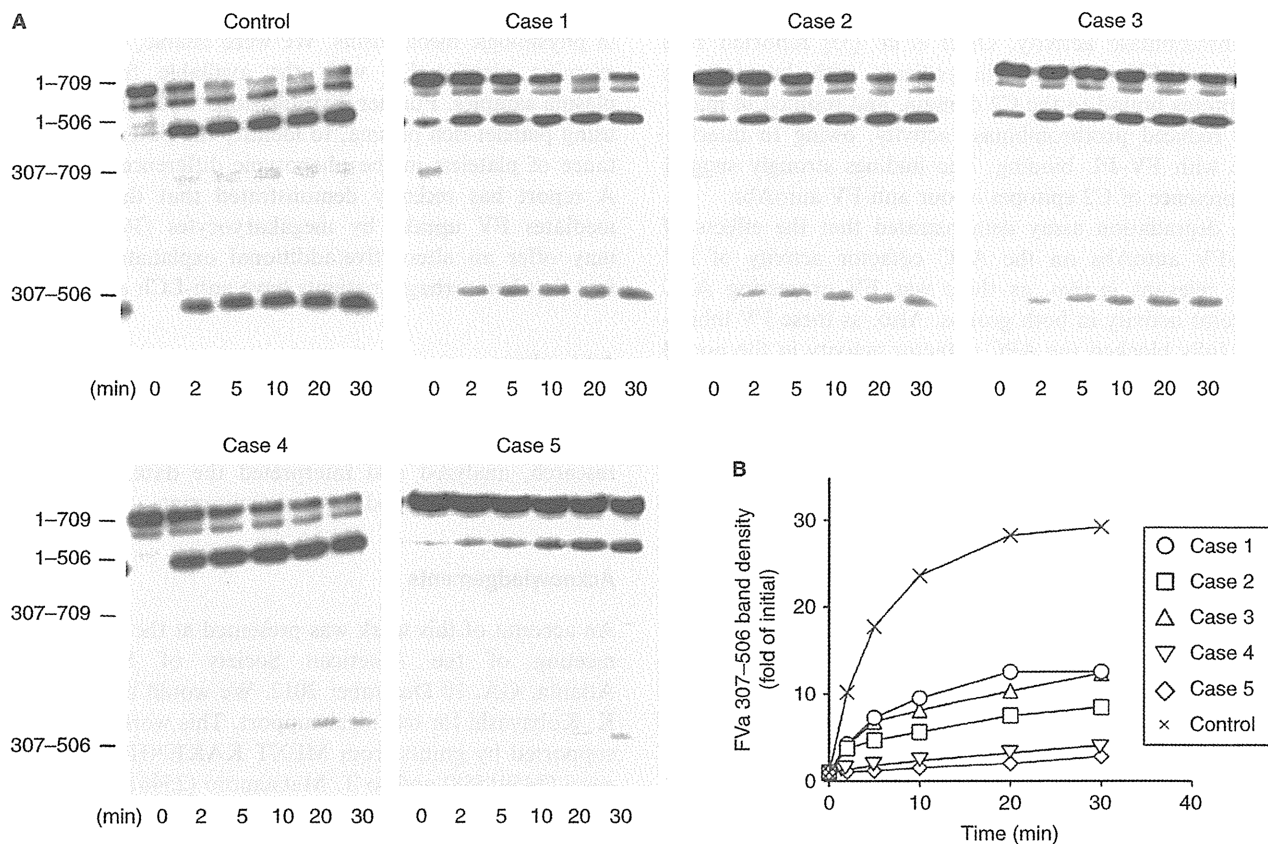

Fig. 7. Activated protein C (APC)-catalyzed proteolytic cleavage of the FVa heavy chain in the presence of anti-FV autoantibodies (autoAbs) from the non-B group. Mixtures of FV (8 nM) and anti-FV autoAbs (30 μg mL^{-1}) from the non-B group were incubated with thrombin (30 nM) for 5 min, and the reaction was terminated by the addition of hirudin. FVa (0.5 nM) from these mixtures was incubated with APC (0.7 nM), protein S (30 nM) and phospholipid (20 μM) for the indicated times. Samples were analyzed on 8% gels, and this was followed by western blotting with an anti-FV mAb. Band densities of the 307–506 fragments were measured by quantitative densitometry. Individual band densities at time zero were regarded as initial, and those obtained at the indicated times were expressed as fold of initial. All experiments were performed at least three separate times, and representative data are shown.

significantly inhibited FV–PL binding, whereas this was unaffected by non-B inhibitors – consequently, prothrombinase activity was completely depressed in the presence of B group antibodies, but was only modestly depressed in the presence of non-B inhibitors; and (iv) anti-FV autoAbs from the non-B group inhibited APC-mediated cleavage of FVa at Arg306, but not at Arg506, and also impaired the APC cofactor activity of FV, consistent with APCR in the non-B group. Overall, the findings demonstrated that negligible or severely reduced prothrombinase activity in the B group, as a result of the inhibition of FV(a)–PL binding by anti-FV autoAbs, caused the severe hemorrhagic symptoms in these patients, whereas the modestly reduced prothrombinase activity together with APCR in the non-B group caused the asymptomatic phenotype.

A one-stage clotting assay is commonly used for the measurement of clotting factors, including FV, and activity levels generally correlate with clinical presentation. Earlier reports have shown, however, that this type of assay has limitations for the assessment of coagulation function in patients with acquired coagulation inhibitors. In consequence, a number of global coagulation functional assays have been established and have been utilized for clinical diagnosis [29,30]. We recently reported that CWA and the TGT identified more disordered coagulation function in patients with acquired hemophilia A than in those with congenital severe hemophilia A (FVIII:C of < 1%) [31]. The present data complement those findings, and show that CWA could help to distinguish between the bleeding and non-bleeding phenotypes in patients with A-FV inhibitors. The results highlight the possibility that this technique could provide valuable data for predicting and/or monitoring hemorrhagic symptoms in patients of this type.

The PL-binding site(s) of FV(a) is located on the C1 and C2 domains within the LCh [26,32], and this binding is associated with the procoagulant action of FV as a cofactor for FXa in the prothrombinase complex. We found that the anti-FV autoAbs in our B group reacted with the LCh, although the precise C2 epitope remains to be identified. Furthermore, these autoAbs inhibited

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FV–PL binding by 60–90%, and markedly depressed prothrombinase activity. Ortel *et al.* [33] reported that their anti-FV inhibitors in patients with hemorrhagic symptoms bound to the C2 domain, and resulted in markedly reduced prothrombinase activity, owing to interference with FV–PL binding. The findings strongly suggest the presence of C2 epitopes in our anti-FV autoAbs.

A degradation assay demonstrated that the effects of anti-FV autoAbs on the APC cofactor activity of FV were relevant *in vivo*, as there was FV expressing APC cofactor activity in both groups. Also, as these FV inhibitors fully blocked the APC cofactor activity in the non-B group, FXa generation increased to the levels observed without FV, such as in cases 1–3, whereas in cases 4 and 5 it increased to the levels obtained without APC. As two autoAbs contained neither anti-PC nor anti-PS autoAbs (data not shown), the FVa–anti-FV autoAb complexes might indirectly affect the association of FVIIIa with APC/PS, but the precise reason is unclear. Although the APC cofactor activity of FV is PL-dependent [34], the autoAbs in the B group that impaired PL binding did not significantly affect the APC cofactor activity. This contradiction may raise the possibility that, because the autoAbs fully block the FV-related procoagulant process, the action of APC cofactor activity may be unlikely to be relevant.

The anti-FV autoAbs in the non-B group significantly delayed APC-mediated cleavage of FVa at Arg306 alone. In addition, these antibodies impaired the APC cofactor activity of FV in FVIIIa inactivation. Many studies on the R506Q mutation (FV Leiden) [13,14] have shown that defective APC cofactor activity in these instances is related to the inhibition of APC-mediated cleavage of FV at Arg506. In the non-B group, APC-mediated proteolytic patterns were different between FV and FVa. Inhibition of cleavage of FV at Arg506 was evident, whereas proteolysis of FVa at Arg306 was delayed with inhibitors. The APC-binding site(s) on the FVa HCh remains to be identified, however, and studies to localize these molecular interactions are now in progress.

Kalafatis *et al.* [19] reported severe thrombotic manifestations in one patient with an A-FV inhibitor. This autoAb recognized a conformational epitope on the entire FV molecule, and, as in our present study, the inhibitor was associated with APCR, owing to impaired APC cofactor activity and restricted APC-mediated cleavage at Arg506 and Arg306 in FVa. However, inhibition of APC-mediated cleavage of FVa in our patients was related to impaired cleavage at Arg306. Unlike the individual described by Kalafatis [19], our patients were asymptomatic, and it might be that inhibition of APC-mediated cleavage at both Arg506 and Arg306 of FVa has a more detrimental effect on the normal hemostatic balance, resulting in thrombotic complications.

The role of FV in hemostasis involves activity present in both plasma and platelets [35], and it might be that platelet FV:C is more important than plasma FV:C in physiologic mechanisms. We were unable, however, to examine platelet FV with the available frozen-stored plasma samples. Further investigations would be required, using platelet-rich plasma, to identify the potential importance of platelets in the phenotypic differences observed. A report has recently demonstrated that the FV LCh mediates FV uptake by megakaryocytes [36], and this may offer an alternative/additional explanation for the fact that hemorrhagic patients have anti-LCh antibodies.

Addendum

T. Matsumoto performed experiments, analyzed the data, and produced the figures. K. Nogami designed the research, analyzed and interpreted the data, and wrote the paper. M. Shima edited the manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Contribution of ADAMTS13 to the better cell engraftment efficacy in mouse model of bone marrow transplantation

The adhesive protein von Willebrand factor (VWF) plays an essential role in physiological hemostasis, mediating platelet adhesion and aggregation under high shear stress conditions.[1,2] The VWF-cleaving protease ADAMTS13 precisely down-regulates VWF activity to avoid pathological intravascular thrombosis in the microvasculature, including arterial capillaries, where blood flow typically creates high shear stress.[1-3] Indeed, the functional deficiency of ADAMTS13 is known to cause thrombotic thrombocytopenic purpura, a typical thrombotic occlusion of the microvasculature.[2,4,5] Thus, the proper equilibrium between VWF and ADAMTS13 is necessary for robust microcirculation *in vivo*. In this context, we hypothesized that ADAMTS13 might contribute to better donor cell homing and engraftment in various cell therapy approaches, in which fluent blood flow in the microcirculation system could be critical. To test this hypothesis, we investigated the role of ADAMTS13 on donor cell engraftment using a bone marrow transplantation (BMT) model in ADAMTS13 gene-deleted (*Adamts13 -/-*) mice.

Adamts13 -/- (KO) mice were back-crossed for more than 15 generations to the C57BL/6 background, as described.[6] Wild-type (WT) mice (C57BL/6-background) were purchased from Japan SLC (Shizuoka, Japan). All mice used in this study were 8-12 weeks old with body weights of 25-30 grams. Mouse experiments were performed in accordance with protocols approved by the Ethics Review Committee for Animal Experimentation of Nara Medical University. In the BMT experiment, recipient WT or KO mice were conditioned for cellular transplantation with lethal total body irradiation (TBI: 5.5 × 2 = total 11 Gray) using a cesium irradiator (MBR-1520, Hitachi, Tokyo,

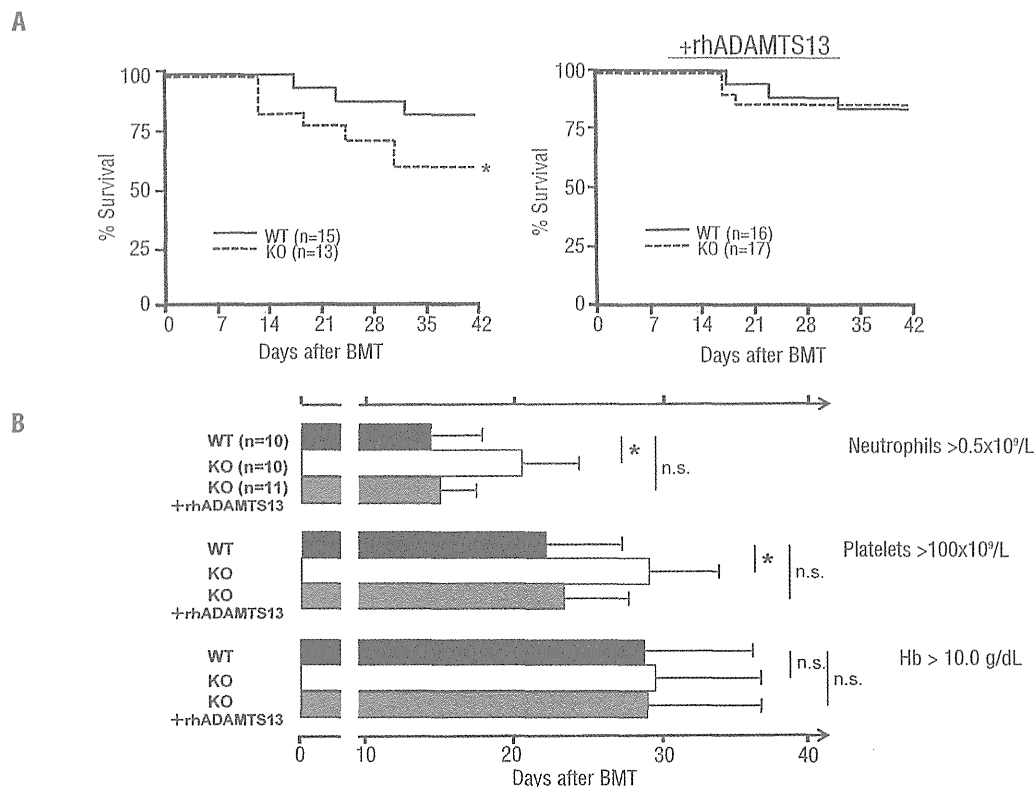

Figure 1. Survival rates and bone marrow suppression of wild-type (WT) or *Adamts13* $^{-/-}$ (KO) mice receiving TBI and subsequent BMT. (A) Kaplan-Meier analysis of survival rates of WT or KO mice receiving TBI and BMT. GFP-positive donor bone marrow cells (5×10^6/mouse) were transplanted to sex-matched WT (n=15) or KO (n=13) mice within 6 hours after TBI (total dose of 11 Gray/mouse) via tail vein. In some indicated experiments (right panel), recombinant human ADAMTS13 (rhADAMTS13; 5 µg/mouse, equivalent to 200 U/kg) was added to the donor bone marrow cell suspension prior to cellular transplantation. Significance of survival studies was quantified using Kaplan-Meier analysis and log rank tests. Note that the survival rate of KO mice began declining significantly (*P<0.05) at Day 14 of BMT, as compared to the WT mice (left panel; WT: 100% *vs.* KO: 76.9%). Following bolus administration of rhADAMTS13, this impaired survival rate in KO mice (WT: 81.0% *vs.* KO: 61.5% at Day 35) improved and became nearly indistinguishable from WT mice (see right panel). (B) Sequential peripheral blood analysis of WT or KO mice after TBI and BMT. Recipient mice were anesthetized using isoflurane inhalation, and 70 µL of blood was collected from the saphenous vein. Complete blood counts of recipient WT (n=10) or KO (n=10) were determined with an automatic blood cell counter (pocH®-100i: Sysmex, Kobe, Japan) every three days following BMT. Each bar represents the mean ± standard deviation (SD) duration that neutrophils counts were > 0.5×10^9/L, platelet tcounts were > 100×10^9/L, or hemoglobin values (Hb) were > 10.0 g/dL. Differences between groups were evaluated by Student's t-test. Note that nadir periods of KO mice are significantly (*P<0.05) longer than those of WT mice with regard to neutrophil and platelet counts (WT: 14.4 ± 3.3 and 22.4 ± 3.5 days *vs.* KO: 20.2±3.8 and 28.5±4.8 days, respectively), while no differences between these 2 groups are seen in Hb (WT: 28.2 ± 7.8 days vs. KO: 29.7 ± 7.5 days). These nadir period prolongations were improved by rhADAMTS13 (n=11) to an extent comparable to those of WT (n.s.: not significant).

Japan). Bone marrow cells to be transplanted were collected from femurs and tibias of donor green fluorescence protein (GFP) mice[7] (purchased from Japan SLC: C57BL/6-background), as described.[8] After removing the red blood cells by lysing with Tris-buffered ammonium chloride, suspended donor bone marrow mononuclear cells were transplanted into irradiated sex-matched recipient mice via tail vein. In some indicated experiments, recombinant human ADAMTS13 (rhADAMTS13), which was prepared as previously described,[9] was added to the donor bone marrow cell suspension prior to cellular transplantation. The VWF-cleaving activity of rhADAMTS13 was determined by *in vitro* FRETS-VWF73 assay.[10]

Kaplan-Meier analysis showed that the mean survival rate of KO mice receiving TBI and subsequent BMT was significantly lower than that of WT mice starting at Day 14 after BMT, and recombinant ADAMTS13 restored the survival rate of KO mice to that of WT mice (Figure 1A). Since all WT and KO mice that underwent TBI without BMT died within 21 days (*results not shown*), the mortality rates under our experimental conditions most likely depended upon the cell engraftment efficacy during BMT and indicate an important contribution of ADAMTS13 in this regard. Indeed, peripheral blood analysis following BMT revealed the longer nadir period in KO mice with regard to neutrophils and platelets (Figure 1B), which was shortened significantly by recombinant ADAMTS13, with the resulting nadir periods comparable to those of WT mice (Figure 1B).

In addition to the above long-term observation experiment, some recipient mice were sacrificed at Days 1, 7, and 14 after BMT to check the extent of donor cell engraftment to the bone marrow and to assess the pathohistological conditions of major organs. After removing the red blood cells, the recipients' bone marrow was collected from the femurs and tibias and used to assess donor cell engraftment efficacy based on the percentage of GFP-positive cells rela-

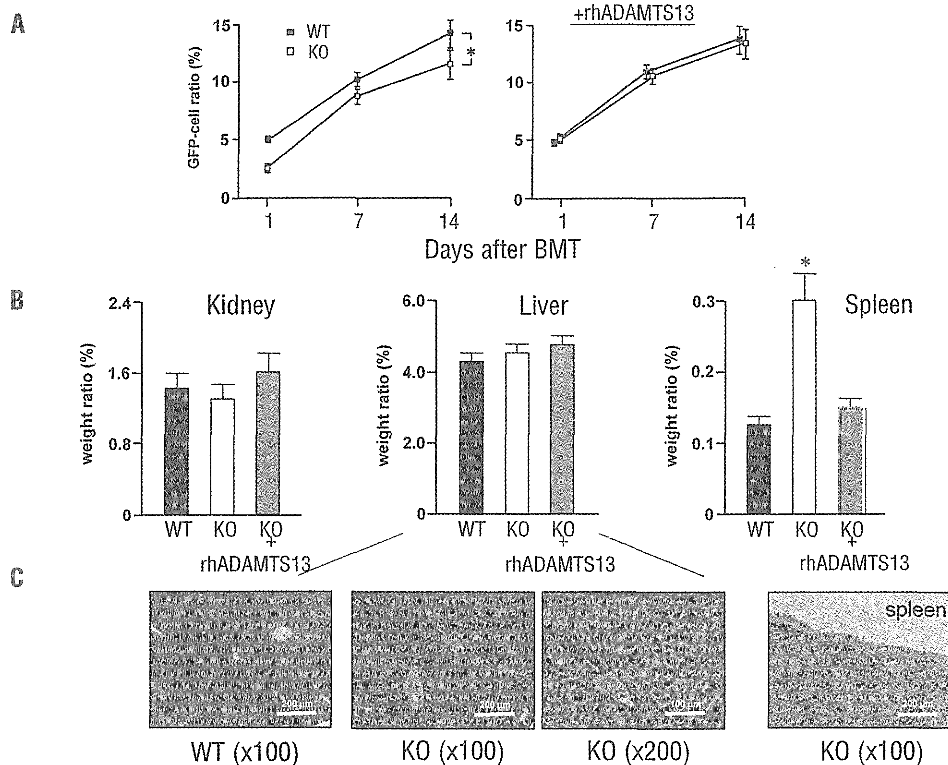

Figure 2. Bone marrow analysis and pathohistological studies in WT or KO mice that received TBI and subsequent BMT. These series of experiments, in which the recipient mice were sacrificed at Days 1, 7, and 14 after BMT (n=5 each), were performed independently of the long-term observation experiments in Figure 1. (A) Flow cytometric analysis of bone marrow cells from WT or KO mice that received TBI and BMT. Each data point represents the average ± SD of "GFP-cell ratio," the percentage of GFP-positive cells relative to total mononuclear cells in bone marrow. Note that a significant (*$P < 0.05$) reduction of donor GFP-positive cells in KO mice is already seen at Day 1 and continues throughout the observation period. In terms of cell propagation in KO mouse marrow, GFP-cells gradually increased in a time-dependent manner similar to that of WT (left panel). This GFP-cell reduction in KO mice was eliminated by rhADAMTS13 (see right panel). (B) Macroscopic findings of major organs in mice sacrificed at Day 7. Each bar represents the average ± SD of "weight ratio," the percentage of each organ weight (kidney, liver, or spleen) relative to total mouse body weight. In terms of macroscopic appearance, no particular differences were seen between WT and KO mice, except for a larger spleen in KO mice (*results not shown*). In fact, the calculated weight ratio confirmed the significant (*$P < 0.05$) splenomegaly in KO mice, which was eliminated by rhADAMTS13 administration. Mild splenomegaly, the extent of which was improved, remained in the corresponding Day 14 samples of KO mice (*results not shown*). (C) Microscopic findings of liver or spleen in mice sacrificed at Day 7. Images displayed are representative of 5 independent mouse samples. The liver samples with hematoxylin-eosin staining (×100 or ×200: original magnification) demonstrate slight dilation of the portal and central veins as well as mild sinusoidal congestion in both WT and KO mice, albeit less pronounced in WT mice. KO mouse livers do not exhibit either typical thrombotic legions in micro-vessels or SOS-lesions. As consistent with macroscopic splenomegaly, mild congestion and external capsule hypertrophy are observed in spleen of KO mice. These microscopic findings are basically similar to the corresponding Day 14 samples (*results not shown*).

tive to total mononuclear cells using flow cytometer (BD LSR-II: Nippon Becton Dickinson Company Ltd., Tokyo, Japan). Consistent with the findings in the peripheral blood, flow cytometric analysis of recipient bone marrow revealed the reduction of donor GFP-positive cells in KO mice that was already significant at Day 1 after BMT (Figure 2A). The population of GFP-positive cells in the bone marrow of KO mice expands gradually in a time-dependent manner similar to that of WT mice (Figure 2A), suggesting that ADAMTS13 is likely to play a role in the initial donor cell homing rather than cell propagation in the bone marrow cell engraftment. Thus, our results could verify the initial hypothesis that ADAMTS13 may contribute to better donor cell homing to the target recipient marrow, a process that requires fluent blood flow in the microvasculature including arterial capillaries.

Thrombotic microangiopathy (TMA) is a well-recognized serious complication of BMT, especially in the liver in the form of sinusoidal obstruction syndrome (SOS), and is known to be associated with functional ADAMTS13 deficiency.[11] Our histological studies, however, have only confirmed mild congestion and sinusoidal dilatation in the liver as well as significant splenic enlargement and congestion in KO mice, without typical thrombotic or SOS lesions of microvessels (Figure 2B and C). These histological findings may be consistent with possible portal hypertension, perhaps reflecting transient occlusion of the microvasculature by enhanced leukocyte plugging or platelet micro-aggregate formation that may occur in systemic microcirculation. Thus, the reduced local microcirculation could result in the poor donor cell homing to bone marrow that was observed in KO mice. Indeed, some clinical symptoms of TMA with functional deficiency of ADAMTS13 are known to be labile and variable,[5] suggesting the existence of transient microvasculature occlusion that cannot be reproducibly demonstrated in final tissue sample sections.

Recent mouse model studies by us and others demonstrated that proper functional regulation of VWF by ADAMTS13 significantly ameliorates the severity of fatal arterial thrombosis in conditions such as cerebrovascular accident or myocardial infarction.[12-15] ADAMTS13 reduces VWF-dependent platelet microaggregate formation as well as inflammatory responses such as leukocyte accumulation at ischemic sites, both of which may result in local microvasculature occlusion.[5] Thus, this property of ADAMTS13 can protect against impaired microcirculation *in vivo*, and may also contribute to better donor cell homing and engraftment in various cell therapy approaches that require fluent blood flow in the microvasculature.

In conclusion, our results illustrate that the regulation of VWF-mediated thrombotic or inflammatory responses by ADAMTS13 may contribute to the improved systemic microcirculation critical for efficient donor cell homing and engraftment in BMT, suggesting a clinical therapeutic potential of ADAMTS13 in cell therapy approaches.

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Key words: bone marrow transplantation, cell engraftment, ADAMTS13, efficacy, mouse model.

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Letters to the Editor

Late vitamin K deficiency bleeding in an infant born at a maternity hospital

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Kaga *et al.* described an additional case of late vitamin K deficiency bleeding (VKDB) in an infant with inadequate prophylactic vitamin K.[1]

In the fifth Japanese nationwide survey of late VKDB in infancy performed between January 1999 and December 2004, 71 cases of late VKDB were reported.[2] At that time, a three-dose oral regimen with 2 mg of vitamin K was recommended in Japan.[3] The consensus on prevention of late VKDB by vitamin K prophylaxis is well known, but late VKDB is still occurring, with insufficient vitamin K treatment noted even in healthy infants: 42/71 infants (59%) had inadequate vitamin K (of the three standard doses required, it was given only once in 27 cases, and only twice in 15 cases) in the fifth nationwide survey in Japan.[2]

Recently, the current status of vitamin K treatment for infants has been modified as follows: oral vitamin K prophylaxis at birth with 2 mg phytomenadione, followed by weekly oral vitamin K prophylaxis; 2 mg should be given by the parents until 3 months of age (1 month of age in formula-fed infants).[4] Although weekly oral vitamin K prophylaxis for infants effectively reduced the incidence of late VKDB,[5,6] a repeated oral dose regimen may not be practical because of poor patient compliance.[7] Many parents lack basic knowledge about the importance and safety of vitamin K.[8]

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The regimen of weekly 1 mg vitamin K treatment for 3 months in Denmark, where compliance was high, resulted in 100% protection against VKDB.[6] In order to ensure successful vitamin K treatment for infants, there is an urgent need to inform parents about the risk and benefit of safe vitamin K treatment, as well as to provide them with perinatal facilities.

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