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Endothelial cell-derived von Willebrand factor, but not platelet-derived, promotes atherosclerosis in Apoe-deficient mice

Prakash Doddapattar[1], **Nirav Dhanesha**[1], **Mehul R. Chorawala**[1], **Chandler Tinsman**[2], **Manish Jain**[1], **Manasa K. Nayak**[1], **Janice M. Staber**[2], and **Anil K. Chauhan**[1]

[1]Department of Internal Medicine, University of Iowa, Iowa City, IA

[2]Stead Family Department of Pediatrics, University of Iowa, Iowa City, IA

Abstract

Objective—von Willebrand factor (VWF) is synthesized by endothelial cells and megakaryocytes, and is known to contribute to atherosclerosis. *In vitro* studies suggest that platelet-derived VWF (Plt-VWF) is biochemically and functionally different from endothelial cell-derived VWF (EC-VWF). We determined the role of different pools of VWF in the pathophysiology of atherosclerosis.

Approach and Results—Using bone marrow (BM) transplantation, we generated chimeric Plt-VWF, EC-VWF, and Plt-VWF mice lacking ADAMTS13 in platelets and plasma (Plt-VWF/ADAMTS13$^{-/-}$) on apolipoprotein-deficient (*Apoe*$^{-/-}$) background. Controls were chimeric *Apoe*$^{-/-}$ mice transplanted with BM from *Apoe*$^{-/-}$ mice (wild-type; WT) and *Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice transplanted with BM from *Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice (VWF-KO). Susceptibility to atherosclerosis was evaluated in whole aortae and cross sections of the aortic sinus in female mice fed a high fat "Western" diet for 14 weeks. VWF-KO, Plt-VWF, and Plt-VWF/ADATS13$^{-/-}$ mice exhibited reduced plaque size characterized by smaller necrotic cores, reduced neutrophil and monocytes/macrophages content, decreased MMP9, MMP2, and CX_3CL1-positive area and abundant interstitial collagen ($P<0.05$ versus WT or EC-VWF mice). Atherosclerotic lesion size and composition was comparable between WT or EC-VWF mice. Together these findings suggest that EC-VWF, but not Plt-VWF, promotes atherosclerosis exacerbation. Furthermore, intravital microscopy experiments revealed that EC-derived VWF, but not Plt-derived VWF, contributes to platelet and leukocyte adhesion under inflammatory conditions at the arterial shear rate.

Conclusions—EC-VWF, but not Plt-VWF, contributes to VWF-dependent atherosclerosis by promoting platelet adhesion and vascular inflammation. Plt-VWF even in the absence of ADAMTS13, both in platelet and plasma, was not sufficient to promote atherosclerosis.

Keywords

von Willebrand factor; plaque; platelet; and endothelial cell

Corresponding author: Anil K. Chauhan, Ph.D., University of Iowa, Department of Internal Medicine, Division of Hematology, Oncology and Blood & Marrow Transplantation, 3188 Medical labs, Iowa City, Iowa-52242, Telephone: 319-335-6525, Fax: 319-353-8383, anil-chauhan@uiowa.edu.

Disclosures

None.

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Introduction

von Willebrand factor (VWF) is a large multimeric protein that plays an important role in hemostasis and thrombosis. VWF is stored as ultra large (ULVWF) multimers or high molecular weight multimers (HMWM) in endothelial Weibel-Palade bodies and platelet α-granules.[1] Upon secretion into circulation and under shear stress, anchored hyperactive ULVWF multimers on the endothelium are cleaved by ADAMTS13 (A Disintegrin And Metalloprotease with Thrombospondin type I repeats-13) into smaller less active VWF multimers that support hemostasis and thrombosis.[2] VWF present in plasma is derived from both endothelial cells (~80–85% via a constitutive secretory pathway or regulated secretion in response to secretagogues such as TNF and histamine) and platelets (~15–20 % from activated platelets).[3, 4]

Several murine studies suggest that VWF/ADAMTS13 axis modulates vascular inflammation in addition to thrombosis in experimental models.[5–13] For example, ADAMTS13 deficiency in mice exacerbates myocardial, and brain ischemia/reperfusion injury by promoting thrombosis and inflammatory processes,[7, 8, 11, 14] whereas VWF deficiency had a protective effect.[7, 11, 14, 15] Similarly, ADAMTS13 deficiency in mice exacerbates atherosclerosis,[9, 10, 16] whereas VWF deficiency reduces atherosclerosis in mice and pigs.[9, 12, 17] Although the role of VWF in atherosclerosis is well established, the relative contribution of platelet-derived VWF (Plt-VWF) versus endothelial cell-derived VWF (EC-VWF) in atherosclerotic lesion progression is not yet elucidated. Notably, several *in vitro* studies suggest that there are biochemical and functional differences between platelet-derived VWF (Plt-VWF) and endothelial cell-derived VWF (EC-VWF). For example, Plt-VWF differs in glycosylation profile, particularly with a reduction in N-linked sialic acid expression when compared to EC-VWF.[18] Although platelets contain ADAMTS13, the authors suggested that due to different glycosylation profile, Plt-VWF is resistant to ADAMTS13 cleavage.[18] In contrast, other *in vitro* studies have shown that Plt-derived ULVWF multimers are only observed when platelets are activated or lysed in the presence of EDTA.[19, 20] There is also a functional difference between EC-VWF and Plt-VWF. Although binding affinities to collagen are comparable between EC-VWF and Plt-VWF, Plt-VWF binds with higher affinity to the surface of thrombin-stimulated platelets when compared with EC-VWF.[21, 22] On the other hand, EC-VWF binds with higher affinity to platelet GPIbα when compared to Plt-VWF.[22]

Herein, we sought to determine the relative contribution of platelet-derived VWF versus endothelial cell-derived VWF pool in the pathophysiology of atherosclerosis. Using reciprocal bone marrow transplantation (BMT), we generated chimeric Plt-VWF and EC-VWF mice on the Apoe-deficient background. We found that EC-VWF, but not Plt-VWF, contributes to atherosclerotic lesion progression by promoting platelet adhesion and vascular inflammation.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

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Results

Characterization of chimeric mice expressing Plt-derived VWF and EC-derived VWF on *Apoe*$^{-/-}$ background

In the remainder of the manuscript: 1) irradiated *Apoe*$^{-/-}$ mice reconstituted with BM from *Apoe*$^{-/-}$ donors (*Apoe*$^{-/-}$-BM→ *Apoe*$^{-/-}$ mice) are controls and are referred as wild-type (WT) mice (source of VWF: endothelial cells and platelets), 2) irradiated *Apoe*$^{-/-}$ mice reconstituted with bone marrow from *Vwf*$^{-/-}$*Apoe*$^{-/-}$ donors (*Vwf*$^{-/-}$*Apoe*$^{-/-}$-BM→ *Apoe*$^{-/-}$ mice) are referred as EC-VWF mice (express VWF in endothelial cells, but lack VWF in platelet), 3) irradiated *Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice reconstituted with BM from *Vwf*$^{-/-}$*Apoe*$^{-/-}$ donors (*Vwf*$^{-/-}$*Apoe*$^{-/-}$-BM→ *Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice) are referred as VWF-KO mice, 4) irradiated *Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice reconstituted with bone marrow from *Apoe*$^{-/-}$ donors (*Apoe*$^{-/-}$-BM→ *Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice) are referred as Plt-VWF mice (have VWF in platelets, but lack VWF in endothelial cells), and 5) irradiated *Adamts*13$^{-/-}$*Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice reconstituted with bone marrow from *Adamts*13$^{-/-}$*Apoe*$^{-/-}$ donors (*Adamts*13$^{-/-}$*Apoe*$^{-/-}$-BM→ *Adamts*13$^{-/-}$*Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice) are referred as Plt-VWF/ADAMTS13$^{-/-}$ mice (ADAMTS13 deficient in plasma and platelet plus lack VWF in endothelial cells) (Figure 1A). Complete blood counts were comparable suggesting that BMT did not affect the number of BM-derived blood cells (Table SI). To confirm successful engraftment of bone marrow, the relative content of VWF in plasma and platelets was quantified by ELISA. Plasma VWF levels were comparable between WT mice (414.5 ± 53.5 %,) and EC-VWF mice (439.9 ± 29.0 %, Figure 1B). Notably, plasma VWF levels in *Apoe*$^{-/-}$ mice are relatively higher to WT levels. No detectable levels of plasma VWF were found in VWF-KO mice, whereas a minor amount of plasma VWF (Figure 1B) was found in Plt-VWF (6.7 ± 1.1 %) and Plt-VWF/*Adamts*13$^{-/-}$ mice (5.6 ± 0.9 %). Plt-derived VWF levels were comparable between WT, Plt-VWF mice and Plt-VWF/ADAMTS13$^{-/-}$ mice (Figure 1C). No detectable level of Plt-derived VWF was found in VWF-KO or EC-VWF mice (Figure 1C).

EC-derived VWF, but not platelet-derived VWF, exacerbates atherosclerosis in aorta and aortic sinus

To define the relative contribution of Plt-derived VWF versus EC-derived VWF pool in the pathophysiology of atherosclerosis, EC-VWF and Plt-VWF female mice with controls (WT and VWF-KO) were fed a high-fat “Western” diet for 14 weeks. We measured total cholesterol and triglyceride levels in all the groups fed a high fat “Western” diet for 14 weeks. Total cholesterol and triglyceride levels were comparable among groups (Figure SI). Next, we compared the extent of atherosclerotic lesion progression in whole aortae by staining with Oil Red O and quantifying *en face* lesion area. Lesion areas were comparable between EC-VWF (22.8 ± 2.3 %) and WT mice (21.8 ± 1.8 %; Figure 2AB). Similar to previous reports,[9, 12] VWF-KO mice exhibited significantly reduced lesions area (12.7 ± 1.1 %, $P<0.05$) when compared with either EC-VWF or WT mice. Atherosclerotic lesion area was comparable between Plt-VWF (14.7 ± 1.3 %) and VWF-KO mice (Figure 2AB). These results suggest that EC-derived VWF, but not Plt-derived VWF, contributes to accelerated atherosclerosis in the aorta. Next, we determined whether Plt-VWF, in the absence of ADAMTS13 in plasma and platelets, is able to promote atherosclerosis

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comparable to EC-VWF. Lesion areas were comparable in Plt-VWF mice (14.7 ± 1.3%) and Plt-VWF/ADAMTS13$^{-/-}$ mice (11.4 ± 1.0 %) but significantly decreased when compared to WT mice (21.8 ± 1.8 %) or EC-VWF mice (22.8 ± 2.3 %, P<0.05, Figure 2A and B). This result suggests that Plt-VWF even in the absence of ADAMTS13 was not sufficient to promote atherosclerosis similar to EC-VWF.

Next, we quantified the cross-sectional area of atherosclerotic lesions in the aortic sinus using the VerHoeffs/Van Gieson staining method. We found that the mean lesion areas (in μm^2 or %) in the aortic sinus were comparable between VWF-KO (29.5 ± 2.2 %), Plt-VWF (33.2 ± 1.8 %) and Plt-VWF/ADAMTS13$^{-/-}$ mice (29.3 ± 1.6 %), but significantly decreased when compared to EC-VWF (41.0 ± 1.3 %) or WT mice (45.6 ± 2.6 %, P<0.05, Figure 3 A–C). Furthermore, necrotic area (unstained acellular area, %) was comparable between VWF-KO, Plt-VWF and Plt-VWF/ADAMTS13$^{-/-}$ mice, but significantly decreased when compared to EC-VWF or WT mice (P<0.05, Figure 3D).

EC-derived VWF, but not platelet-derived VWF, promotes vascular inflammation in aortic sinus of Apoe$^{-/-}$ mice

To test the hypothesis that EC-VWF promotes vascular inflammation within atherosclerotic lesions, we quantitated macrophage and neutrophil infiltration within lesions in the aortic sinus by immunohistochemistry. We found that the Mac-3 positive area (μm^2) were comparable between VWF-KO, Plt-VWF and Plt-VWF/ADAMTS13$^{-/-}$ mice, but significantly decreased when compared to EC-VWF or WT mice (P<0.05, N=10–12 mice/group, Figure 4A). Not only was the absolute Mac-3 positive area increased in EC-VWF and WT mice (Figure SII), but the percent of the total lesion area occupied by Mac-3 positive cells was also significantly higher in EC-VWF or WT mice when compared to VWF-KO or Plt-VWF or Plt-VWF/ADAMTS13$^{-/-}$ mice (P<0.05, Figure 4A). Next, we measured neutrophil content within lesions. We observed that the content of Ly6G-positive neutrophils in Apoe$^{-/-}$ mice fed a high fat “Western diet” for 14 weeks is markedly (but not completely) less when compared to Apoe$^{-/-}$ mice fed a high fat “Western diet” for 4 weeks (not shown). However, it was still possible to quantify Ly6G-positive cells among all groups. The accumulation of Ly6G-positive cells was comparable between WT and EC-VWF mice but significantly higher when compared with VWF-KO or Plt-VWF or Plt-VWF/ADAMTS13$^{-/-}$ mice (Figure SIII). Next, we measured the interstitial collagen content. Picrosirius red staining of lesions in the aortic sinus revealed similar collagen content in EC-VWF and WT mice but significantly decreased when compared with VWF-KO or Plt-VWF or Plt-VWF/ADAMTS13$^{-/-}$ mice (Figure 4B).

Next, we analyzed other histological features of vascular inflammation including MMP9, MMP2, chemokine CX_3CL1 (known to contribute to platelet adhesion via VWF) and P-selectin, all known modulators of leukocyte infiltration within lesions in the aortic sinus by immunohistochemistry. We found that MMP9-positive, MMP2-positive, and CX_3CL1-positive area were comparable between VWF-KO, Plt-VWF and Plt-VWF/ADAMTS13$^{-/-}$ mice, but significantly reduced when compared to EC-VWF or WT mice (P<0.05, Figure 5). Although there was a trend towards a decreased P-selectin-positive area in VWF-KO, Plt-VWF and Plt-VWF/ADAMTS13$^{-/-}$ mice when compared with WT or EC-VWF mice, it

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was not statistically significant (Figure 5). Together, these results suggest that EC-VWF by promoting vascular inflammation contributes to atherosclerosis exacerbation.

EC-derived VWF, but not platelet-derived VWF promotes platelet and leukocyte adhesion to activated endothelium of Apoe$^{-/-}$ mice

Platelets are known to recruit leukocytes and play a key role in the onset and progression of atherosclerosis.[2324] To define the relative contribution of Plt-derived VWF versus EC-derived VWF pool in recruiting quiescent platelets and leukocytes *in vivo*, we quantified platelet and leukocyte adhesion under the inflammatory condition at the arterial shear rate. Chimeric mice fed a chow diet were challenged with TNF, and the common carotid artery and carotid bifurcation (atheroprone) were visualized for platelet and leukocyte adhesion after 3.5 hours by intravital microscopy. We found that the number of adherent platelets (adherent for >30 s) and leukocytes were comparable between Plt-VWF-KO, VWF-KO and Plt-VWF/ADAMTS13$^{-/-}$ mice, but significantly decreased when compared to WT or EC-VWF mice (Figure 6). The number of adherent platelets and leukocytes were comparable between WT and EC-VWF mice. These results suggest that EC-derived VWF, but not Plt-derived VWF, contributes to platelet and leukocyte adhesion under inflammatory conditions.

Discussion

The results presented herein provide novel insights on the relative importance of different pools of VWF in the pathophysiology of atherosclerosis. The key findings of the study are: 1) EC-VWF, but not Plt-VWF, contributes to VWF-dependent atherosclerosis most likely by promoting platelet adhesion and vascular inflammation. 2) Plt-VWF even in the absence of ADAMTS13 in the platelets and plasma was not able to promote atherosclerosis.

The first evidence on the role of VWF in atherosclerosis was demonstrated in 1978 utilizing pigs with von Willebrand's disease (VWD).[17] These findings were further confirmed at the genetic level using either *Vwf*$^{-/-}$*LDLR*$^{-/-}$ or *Vwf*$^{-/-}$*Apoe*$^{-/-}$ mice,[9, 12] suggesting a protective effect of complete VWF deficiency in atherosclerotic lesion development. However, the relative contribution of different pools of VWF in the pathophysiology of atherosclerosis was never investigated. We determined the differential role of Plt-VWF and EC-VWF in the progression of atherosclerosis because of functional differences between them. Although binding affinities to collagen are comparable between Plt-VWF and EC-VWF, Plt-VWF binds with higher affinity to the surface of thrombin-stimulated platelets when compared with EC-VWF.[21, 22] Other studies suggested that Plt-derived VWF binds efficiently to activated αIibβ3 and αIIb contributes to atherosclerosis.[22, 23, 25, 26] In contrast, Plt-derived VWF was demonstrated to be less capable of binding to GPIbα when compared with EC-VWF.[22] We and others have recently found that Plt-VWF contributed to transient thrombus growth in experimental models of thrombosis.[27, 28] Based on these studies, we speculated that Plt-VWF might alone be sufficient to promote atherosclerosis exacerbation. To our surprise, we found that EC-VWF, but not Plt-VWF, solely contributes to atherosclerotic lesion progression in both aorta and aortic sinus. We found that atherosclerotic lesions in the EC-VWF were not only larger but more inflammatory (increased Ly6G-positive and Mac3-positive content, increased MMP9 and MMP2 content)

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associated with the larger necrotic area and reduced interstitial collagen when compared to Plt-VWF mice. Furthermore, we found an increase in chemokine fractalkine (CX_3CL1) in the lesions of EC-VWF mice when compared to Plt-VWF mice. CX_3CL1 was shown to mediate platelet adhesion and leukocyte recruitment via endothelial VWF.[29] Together, these studies suggest that EC-VWF, but not platelet VWF, exacerbate atherosclerosis by promoting vascular inflammation.

VWF deficiency in mice results in a defect in Weibel-Palade body formation, which results in lower P-selectin expression and defect in leukocyte recruitment in an experimental model of acute inflammation when fed a chow diet.[30] Is it possible that decreased P-selectin expression in endothelial cells of chimeric Plt-VWF mice, which lack EC-VWF, may mask the effect of Plt-VWF in atherosclerotic lesion development? We think that such a possibility is minimal based on following observations. First, the absence of VWF reduces P-selectin dependent leukocyte rolling in mesenteric venules of mice fed a chow diet but not an atherogenic diet.[12] This result suggests that increased P-selectin synthesis and direct plasma membrane deposition under atherogenic conditions is less dependent on regulated secretion of Weibel-Palade bodies.[12] Second, immunohistochemical staining of the aortic sinus revealed that P-selectin expression was comparable between EC-VWF and Plt-VWF mice fed a high a high-fat “Western” diet for 14 weeks. Third, reduction in the atherosclerotic lesion in P-selectin$^{-/-}$LDLR$^{-/-}$ mice was not observed in female mice fed a high-fat diet for 8 weeks.[31] Herein; we have used female mice fed a high fat “Western” diet for 14 weeks.

Platelets contain ADAMTS13,[20, 32] in addition to ADAMTS13 present in plasma. A previous study suggested that Plt-VWF is resistant to ADAMTS13 cleavage *in vitro* because of different glycosylation profile.[18] Based on these observations, we speculated that in the absence of ADAMTS13, VWF/ULVWF released from platelets might be sufficient to support atherosclerotic lesion progression by promoting leukocyte infiltration. To test this, we transplanted irradiated *Vwf*$^{-/-}$*Adamts13*$^{-/-}$*Apoe*$^{-/-}$ mice with BM from *Adamts13*$^{-/-}$*Apoe*$^{-/-}$ donors so that VWF released after platelet activation is not cleaved by ADAMTS13. To our surprise, we found that atherosclerotic lesion progression in the aorta and aortic sinus was comparable in Plt-VWF/ADAMTS13$^{-/-}$ mice to Plt-VWF or VWF-KO mice. Results from these chimeric studies suggest that under physiological conditions Plt-VWF by itself, even in the absence of ADAMTS13, does not contribute to atherosclerosis.

Platelets bound to the activated endothelium are known to promote vascular inflammation, and thereby play a key role in the pathophysiology of atherosclerosis.[2324] We found that EC-VWF, but not platelet-VWF, markedly increased platelet and leukocyte adhesion in atheroprone carotid vessels under inflammatory conditions. These findings are in agreement with other studies, which have shown that EC-VWF released from activated endothelium can recruit quiescent platelets and leukocytes, though at low shear rate similar to condition that might occur in the regions of aorta and aortic sinus.[6, 13, 33, 34] Another study showed that quiescent platelets adhere to endothelium via endothelial VWF get activated and can support leukocyte rolling at high shear stress *in vitro* via leukocyte P-selectin glycoprotein ligand-1 (PSGL-1) and platelet P-selectin.[35] Activated platelets are known to promote acute inflammation by stimulating endothelium[36] and accelerate atherosclerosis. Several studies suggest that in addition to vascular inflammation, hypercoagulability also contributes to the

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pathophysiology of atherosclerosis.[37, 38] The possibility that endothelial VWF/platelet interactions may promote atherosclerosis exacerbation by enhancing hypercoagulability, in addition to vascular inflammation cannot be ruled out.

Despite animal studies that strongly support the role of VWF in atherosclerosis, the evidence of protective effect of VWD in humans is weak.[39–44] Autopsies showed that VWD patients were not protected from atherosclerosis.[42–44] On the other hand patients with more severe hemophilia A or VWD had fewer atherosclerotic plaques compared to patients with less severe coagulopathy.[45] The possibility that VWD patients have some detectable level of VWF activity in plasma (Ristocetin cofactor activity; ~30–50 IU/dL, whereas the normal range is 50–200 IU/dL) that might be sufficient to promote atherosclerosis cannot be ruled out. We note that there is one study, which demonstrated that patients with type 3 severe VWD (completely lack VWF) are not protected from atherosclerosis. However, the overall plaque burden in the study population was less than 20% most likely because of inclusion of the young subjects (mean age 35 years).[46] More clinical studies are needed to further clarify the role of VWF in promoting atherosclerosis in humans.

In conclusion, the present study identifies that EC-VWF, but not Plt-VWF, as a key player that promotes atherosclerosis in the aorta and aortic sinus of *Apoe*$^{-/-}$ mice, a clinical-relevant model of atherosclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard Abbreviations and Acronyms

VWF	von Willebrand factor
ULVWF	Ultra large von Willebrand factor
ADAMTS13	A Disintegrin And Metalloprotease with Thrombospondin type I repeats-13
Plt-VWF	Platelet-derived von Willebrand factor
EC-VWF	Endothelial cell-derived von Willebrand factor
WT	Wild-type
VWF-KO	VWF-Knock out
BMT	Bone marrow transplantation
TNF	Tumor necrosis factor

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Highlights

- VWF is synthesized in endothelial cells and megakaryocytes.
- Endothelial cell-derived VWF, but not platelet-derived, contributes to VWF-dependent atherosclerosis in apolipoprotein E deficient mice by promoting platelet adhesion and vascular inflammation.
- Platelet-derived VWF even in the absence of ADAMTS13 (both in the platelets and plasma) was not sufficient to promote atherosclerosis in apolipoprotein E deficient mice.

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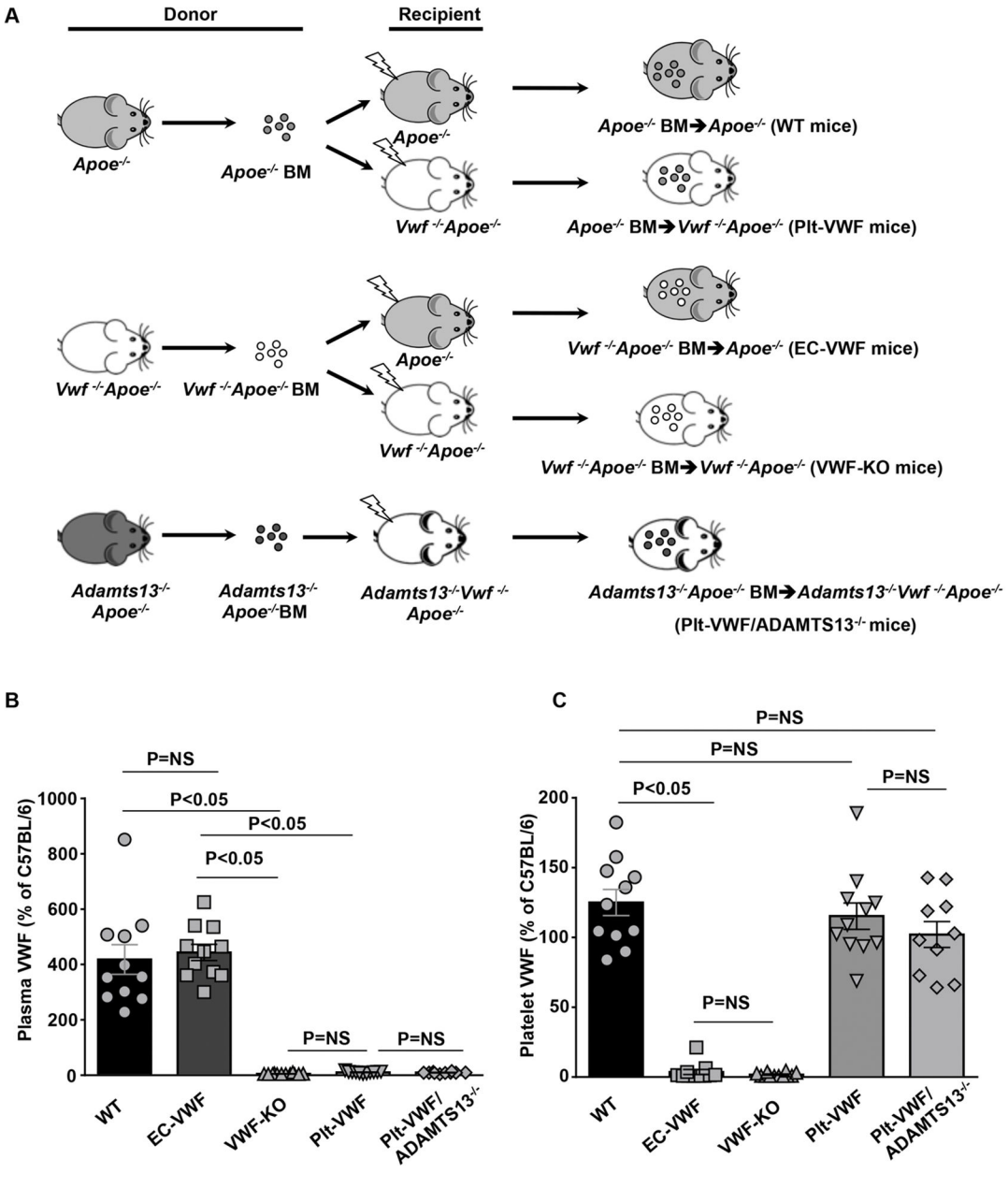

Figure 1.

Scheme and characterization of chimeric mice, which express VWF either in megakaryocytes or endothelial cells**. A,** Schematic representation of BMT protocol. Reciprocal bone marrow (BM) from *Apoe*$^{-/-}$ or *Vwf* $^{-/-}$ *Apoe*$^{-/-}$ mice were transplanted into either *Apoe*$^{-/-}$ or *Vwf* $^{-/-}$*Apoe*$^{-/-}$ recipient mice to generate 4 experimental groups of mice: 1) WT mice: source of VWF is platelets and endothelial cells, control, 2) EC-VWF mice: source of VWF is endothelial cells, 3) VWF-KO mice: lack VWF in platelets and endothelial cells, 4) Plt-VWF mice: source of VWF is platelets, 5) Plt-VWF/ADAMTS13$^{-/-}$ mice: source of VWF is platelets but are deficient for ADAMTS13 in both platelets and plasma. **B,** Plasma VWF levels. N=10–12 mice/group. **C,** Platelet VWF levels. N=10–12

mice/group. Data are presented as mean ± SEM. Statistical analysis: Parametric one-way ANOVA followed by Sidak's multiple comparisons test. NS: non-significant.

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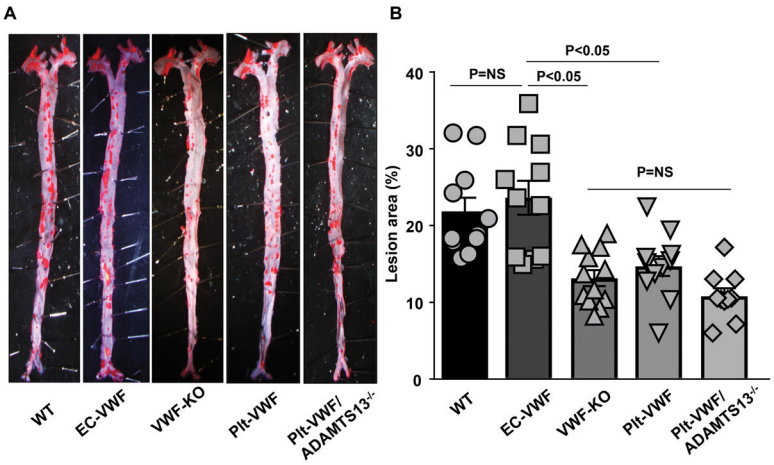

Figure 2.

EC-VWF promotes atherosclerotic lesion formation in the aorta. Female mice from all the groups were fed a high fat "Western" diet for 14 weeks. **A,** Oil red O staining of the representative aortae from each group. **B,** Quantification of en face lesion area. Each dot represents a single mouse. N=10–12 mice/group. Statistical analysis: Parametric one-way ANOVA followed by Sidak's multiple comparisons test. NS: non-significant.

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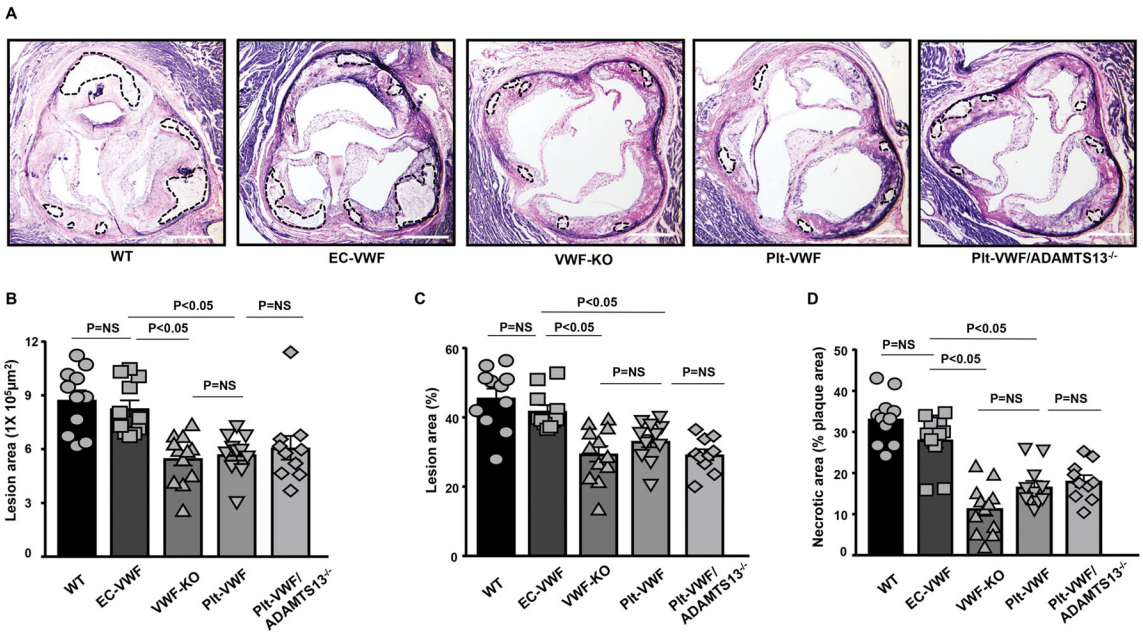

Figure 3.

EC-VWF promotes atherosclerotic lesion formation in the aortic sinus. Female mice from all the groups were fed a high fat “Western” diet for 14 weeks. **A,** Representative photomicrographs of VerHoeffs/Van Geison-stained aortic sinuses. Scale bar = 500 μm. **B & C,** Quantification of the lesion in the cross-section area of aortic sinuses. D, Necrotic core (%) defined as acellular area and demarcated by dashed lines in panel A. Each dot represents a single mouse. N=10–12 mice/group. Statistical analysis: Kruskal-Wallis test followed by uncorrected Dunn’s test. NS: non-significant.

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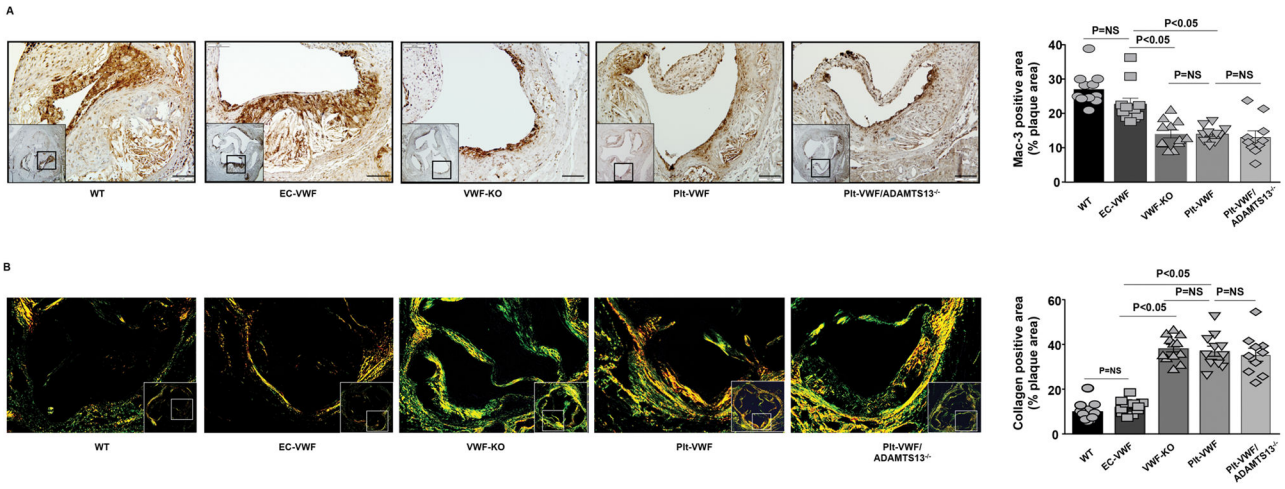

Figure 4.

EC-VWF mice, but not Plt-VWF mice, exhibit increased macrophage infiltration and reduced interstitial collagen within atherosclerotic lesions. **A,** Left-hand side: Higher magnification images showing representative photomicrographs stained for macrophages (Mac-3 positive cells stained as brown) and counterstained with hematoxylin (blue) from the boxed region. Scale bar = 100 μm. Right-hand side: Quantification of macrophages in the cross-section area of aortic sinuses. N=10–12 mice/group. Statistical analysis: Kruskal-Wallis test followed by uncorrected Dunn's test. **B,** Left-hand side: Higher magnification images showing representative photomicrographs stained for collagen from the boxed region and visualized by a polarization microscope. Right-hand side: Quantification of positive collagen area (%) in the cross-section area of aortic sinus. N=10–12/group. Value for each mouse represents a mean of 16 fields from 4 serial sections. Statistical analysis: Parametric one-way ANOVA followed by Sidak's multiple comparisons test. Each dot represents a single mouse. NS: non-significant.

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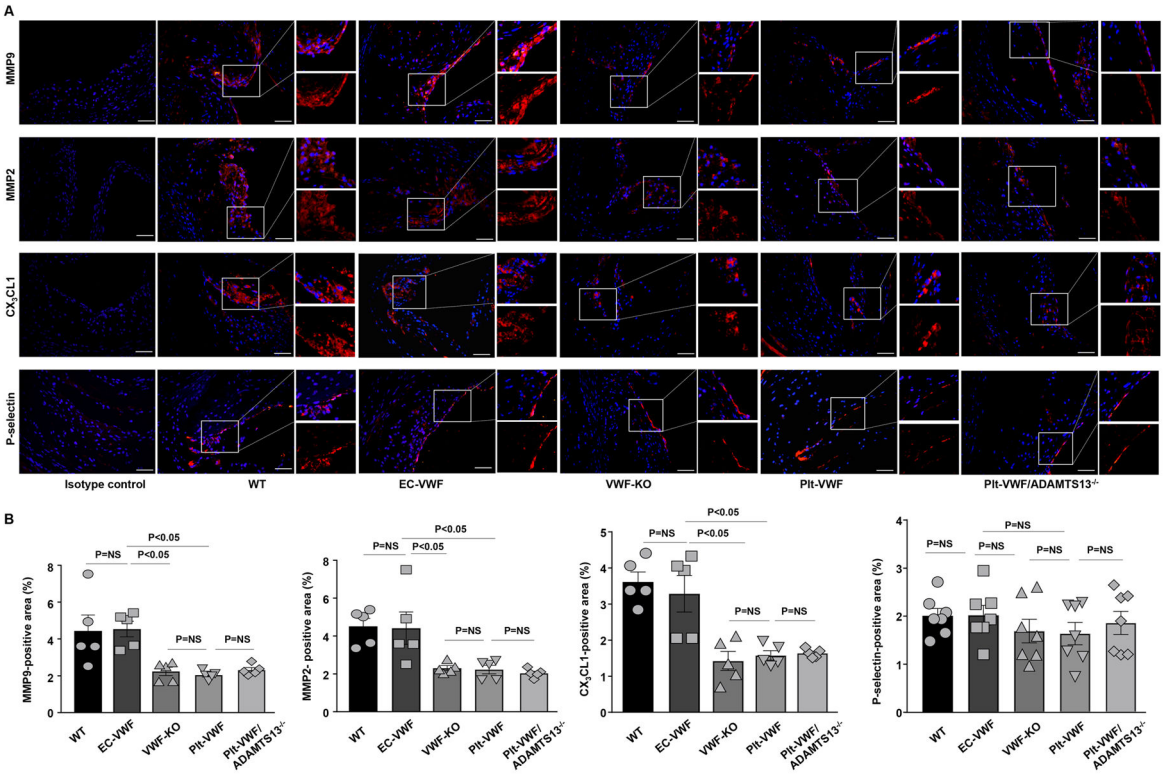

Figure 5.

EC-VWF mice, but not Plt-VWF mice, exhibit increased MMP9, MMP2, and CX_3CL1 expression within atherosclerotic lesions. **A,** Representative immunofluorescence images of sections from different strains of chimeric mice stained with MMP9, MMP2. CX_3CL1, and P-selectin. Nuclei were visualized with DAPI stain. Boxed region is further magnified to show MMP9-positive area, MMP2-positive area, CX_3CL1-positive area and P-selectin-positive area. **B,** Quantification of MMP9-positive area, MMP2-positive area, CX_3CL1-positive area and P-selectin-positive area. Data represent mean ± SEM. Each dot represents a single mouse. Value for each mouse represents a mean from 4 serial sections (each section approximately 60 μm apart). Statistical analysis: Parametric one-way ANOVA followed by Sidak's multiple comparisons test. Scale bar = 50 μm. NS: non-significant.

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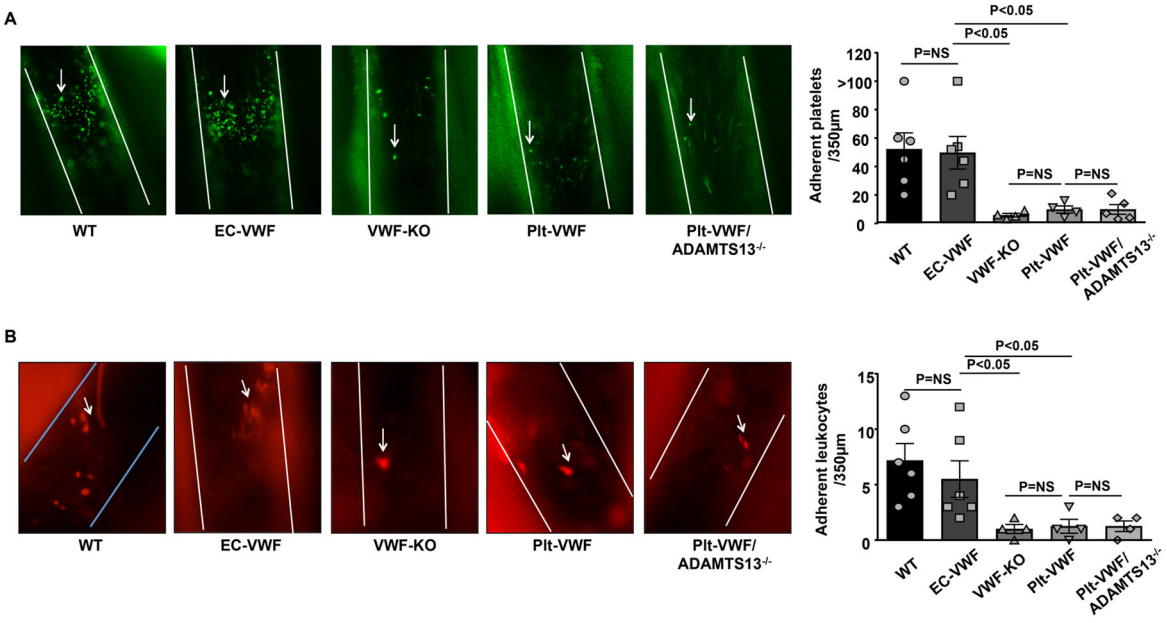

Figure 6.

EC-VWF mice exhibit increased platelet and leukocyte adhesion in carotid vessels under inflammatory conditions. All mice were challenged with recombinant TNF 3.5 hours before visualization of platelet and leukocyte adhesion. **A,** Left-hand side shows representative photomicrographs of adhering platelets (>30 s) at the common carotid artery and carotid bifurcation, a lesion-prone site, as visualized by intravital upright microscope. Magnification= 100X. The right-hand side shows quantification of the number of adherent fluorescent-labeled platelets (calcein green, AM labeled). **B,** Left-hand side shows representative photomicrographs of adhering leukocytes (Rhodamine 6G labeled) at the common carotid artery and carotid bifurcation. The right-hand side shows quantification of adhered leukocytes. Magnification= 200X. White lines delineate the arteries in A and B. Arrows indicate adherent platelet and leukocyte. Data represent mean ± SEM. Each dot represents a single mouse. Statistical analysis: Parametric one-way ANOVA followed by Sidak's multiple comparisons test. NS: non-significant.