HHS Public Access

Author manuscript

J Thromb Haemost. Author manuscript; available in PMC 2022 October 07.

Published in final edited form as:

J Thromb Haemost. 2022 October ; 20(10): 2386–2393. doi:10.1111/jth.15828.

Anti-inflammatory protective effect of ADAMTS-13 in murine arthritis models

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Abstract

Background: Patients with rheumatoid arthritis (RA) have frequent thrombotic events with endothelial dysfunction. Von Willebrand factor (VWF) has been shown to bind neutrophil extracellular traps (NETs) and NETs are part of RA etiology.

Objectives: This study aims to elucidate whether this prothrombotic status exacerbates inflammation in arthritis. Here we focus on the involvement of A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif, member 13 (ADAMTS-13), an enzyme cleaving VWF and its effect on NET deposition and RA development.

Methods: We evaluated the influence of the *Adamts13* gene and recombinant human ADAMTS-13 (rhADAMTS-13) on arthritis in the mouse models of collagen-induced arthritis (CIA). We also assessed VWF and NETs in synovial tissue.

Results: Several *Adamts13*$^{-/-}$ mice developed arthritis, while *Adamts13*$^{+/+}$ siblings did not. Synovial tissue from *Adamts13*$^{-/-}$ showed accumulation of NETs. Treatment of DBA/1 J mice, an arthritis-susceptible strain, with well-tolerated doses of rhADAMT13 reduced arthritis incidence and alleviated the severity of arthritis. Mice treated with rhADAMT13 presented less serum interleukin 6 and less bone erosion determined by micro-computed tomography. The effects on arthritis severity were observed both when administering rhADAMTS-13 prophylactically and also when given after arthritis has developed. In both conditions, rhADAMTS-13 reduced VWF and NET deposition on proliferated synovial tissue evaluated by immunoblotting.

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AUTHOR CONTRIBUTIONS

ShF and DDW conceived and designed the study, analyzed and interpreted data, drafted the manuscript, and gave final approval of the submitted manuscript. ShF, SG, SaF, and LC performed experiments, acquired data, reviewed the manuscript critically, and gave final approval of the submitted manuscript. DDW is the guarantor of this work, had full access to all the data in the study, and took responsibility for the integrity of the data and the accuracy of the data analysis.

CONFLICTS OF INTEREST

DDW has received a grant from Takeda Pharmaceutical Company Ltd. DDW is on the Scientific Advisory Board of Neutrolis a preclinical-stage biotech company, and was a consultant to Takeda Pharmaceutical Company Ltd. ShF, SG, SaF, and LC have nothing to disclose.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Conclusions: Our results demonstrate the inhibitory role of *Adamts13* in murine arthritis and the effectiveness of rhADAMTS-13 treatment. Additionally, this study suggests that deposition of VWF in the synovium and subsequent pathogenic NET retention promotes arthritis. Treatment with rhADAMTS-13 provides a potential therapeutic approach targeting inflammation and pro-thrombotic state in arthritis.

Keywords

citrullination histone; inflammation; neutrophil extracellular trap; rheumatoid arthritis; synovitis; von Willebrand factor

1 | INTRODUCTION

Chronic inflammation in rheumatoid arthritis (RA) has been connected with a prothrombotic state and endothelial dysfunction supporting the development of venous thrombosis,[1] which can potentially shorten the life expectancy of patients with RA. The modulated and unbalanced procoagulant, anticoagulant, and fibrinolysis pathways caused by the systemic inflammation in RA may contribute to these conditions.

It is increasingly recognized that inflammation stimulates thrombosis, and in turn, thrombosis promotes inflammation with functional interdependence of both processes.[2] A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif, member 13 (ADAMTS-13) and leukocytes provide an important contribution to thromboinflammation.[3] It cleaves von Willebrand factor (VWF), a multimeric protein that mediates platelet adhesion and thrombus formation. Mice genetically deficient in ADAMTS-13 have a proinflammatory and prothrombotic phenotype.[3] Furthermore, the administration of recombinant human ADAMTS-13 inhibits inflammation as in myocardial injury[4] and colitis.[5]

The associations of ADAMTS-13 and VWF within RA were suggested previously. Higher levels of plasma VWF have been reported in patients with RA compared to healthy controls.[6] In addition, RA is known as one of the causes of thrombotic microangiopathy, which is characterized by reduced ADAMTS-13 activity.[7]

Given that evidence, it is probable that prothrombotic status causes platelet activation and “the vicious circle”: proinflammatory status leads to prothrombotic status, which in turn exacerbates inflammatory status in RA. We hypothesized that regulating both inflammation and prothrombotic status by targeting ADAMTS-13 could modulate this vicious circle in RA. Therefore, we decided to study the role of ADAMTS-13 in murine arthritis models.

2 | METHODS

2.1 | Animals

ADAMTS13$^{-/-}$ mice[8] on C57BL/6J background were bred in-house and routinely backcrossed to the C57BL/6J background. Wild-type C57BL/6J mice were purchased from the Jackson Laboratory. Littermates derived from heterozygous crosses were used in our genetic studies. DBA/1 J male mice were purchased from Jackson Laboratory. All mice were kept specific pathogen free. Experimental protocols were approved by the

Institutional Animal Care and Use Committee of Boston Children's Hospital (Protocol number: 20-01-4096R).

2.2 | Collagen-induced arthritis and evaluation of arthritis

Mice aged 8 to 12 weeks old were immunized with the emulsion of complete Freund's adjuvant (CFA, catalog number: 7023, Chondrex) and 100 μg of chicken type II collagen (catalog number: 20012, Chondrex) in a 1:1 mixture (total 100 μl) subcutaneously into the base of the tail on day 0.[9] The booster immunization of CII with incomplete Freund's adjuvant (catalog number: 7002, Chondrex) was performed on day 21. The severity of arthritis was evaluated using the following clinical scoring system for each limb: 0, normal; 1, swelling in one finger joint; 2, swelling in more than one finger joint or wrist or ankle joint; 3, swelling in the entire paw; and 4, deformity and or ankylosis. The maximum score was 16 per mouse. Two evaluators (one knew the group allocation, the other did not) independently scored arthritis and arrived at agreements on final scorings.

2.3 | Synchronization of arthritis development by lipopolysaccharide injection

Lipopolysaccharide (LPS; from *E. coli* 055:B5, catalog number: tlrl-pb5lps, InvivoGen, 25 μg/mouse in saline) was administered intraperitoneally on day 25 to synchronize arthritis development.

2.4 | Administration of recombinant human ADAMTS-13

Recombinant human ADAMTS-13 (rhADAMTS-13, kindly provided by Takeda Pharmaceutical Co. Ltd) was injected at 2500 U/kg retro-orbitally once daily in the DBA/1 J mice from day 20 to day 26. For treatment use, rhADAMTS-13 was injected from day 27 to day 33. Sterile saline was the vehicle control.

2.5 | Statistics

Data were described with the median and interquartile range (IQR) for quantitative variables. We assessed the association between variables using Wilcoxon's rank-sum test for quantitative variables. Wilcoxon's signed-rank test was used for corresponding arthritis score pairs. The cumulative incidence of arthritis was estimated with the Kaplan–Meier method and log-rank test. All tests were two-sided, and a p-value <.05 was considered significant. The Bonferroni correction was used for the multiple comparison. All statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software).

See supporting information description on experimental procedures including enzyme-linked immunosorbent assay (ELISA), hematoxylin–eosin (HE) stain, histological evaluation, immunofluorescence microscopy, immunoblotting, micro-computed tomography image acquisition, and analyses.

3 | RESULTS AND DISCUSSION

3.1 | A collagen-induced arthritis model in *Adamts13*$^{+/+}$ and *Adamts13*$^{-/-}$ siblings on C57BL/6 background

We used one injection of the emulsion of type II collagen with CFA and one injection of the emulsion of type II collagen with incomplete Freund's adjuvant due to the pain and distress of two injections of CFA on the C57BL/6J mice (Figure 1A). While previous reports have shown an incidence of arthritis from 0% to 60%[9] in this protocol, none of the 22 wild type (WT) mice had arthritis in our laboratory; however, 20% of *Adamts13*$^{-/-}$ mice (4 out of 20) developed arthritis (Figure 1B).

On day 56, serum interleukin-6 (IL-6) levels of *Adamts13*$^{-/-}$ were higher than those of littermate WT mice (Figure 1C, left panel). No difference in anti-type II collagen IgG antibody was seen between the two groups (Figure 1C, center panel). We also measured the anti-type II collagen IgG2c antibody as C57BL/6 mice produce IgG2c antibodies instead of IgG2a antibodies;[10] however, there was no difference between the two groups (Figure C, right panel).

When evaluated histopathologically, arthritic *Adamts13*$^{-/-}$ mice showed proliferated synovial tissue in the joint space (Figure 1D). Immunofluorescence microscopy revealed citrullinated histone H3 (H3Cit, a marker of neutrophil extracellular traps [NETs]) with DNA in areas within the joint space and on the bone surface in *Adamts13*$^{-/-}$ mice. In contrast, no lesion with H3Cit and DNA was observed in WT mice.

3.2 | The effects of recombinant human ADAMTS-13 treatment on collagen-induced arthritis model in DBA/1 J mice

To evaluate the effect of exogenous ADAMTS-13 on arthritis, we chose DBA/1 J mice susceptible to collagen-induced arthritis (CIA). We injected type II collagen with CFA on day 0 and type II collagen with incomplete Freund's adjuvant on day 21. From day 20 to day 26, rhADAMTS-13 (2500 U/kg) or vehicle (saline) were administered retro-orbitally (Figure 2A). This experiment was done to confirm the effectiveness of rhADAMTS-13 as a preventative strategy. The injection of rhADAMTS-13 reduced arthritis incidence and severity (Figure 2B). The plasma VWF levels increased 2-fold by the induction of arthritis and plasma VWF of rhADAMTS-13-treated mice were significantly lower than those of vehicle-treated mice on day 27 (Figure 2C). The rhADAMTS-13-treated mice demonstrated lower white blood cell count on day 22 than vehicle-treated mice, but no differences in platelet count on day 22 (Figure 2D). The rhADAMTS-13-treated mice had reduced serum IL-6 on day 56, whereas no difference was observed in the serum level of the anti-collagen IgG antibody. Reduced swelling and eroded surface area of bones, as evaluated by the micro-computed tomography (micro-CT), were observed in rhADAMTS-13-treated mice (Figure 2E). Immunofluorescence microscopy revealed H3Cit with DNA, along with neutrophil accumulation in the joint space, on the bone surface in vehicle-treated mice. However, no lesion with citrullinated H3, Ly6G, and DNA was observed in rhADAMTS-13-treated mice even when the mice developed arthritis (Figure 2F). Histological evaluation of joint inflammation, synovial hyperplasia, destruction of

cartilage, and destruction of bone revealed rhADAMTS-13-treated mice had significantly alleviated severity of arthritis compared to vehicle-treated mice (Figure 2F and G). The accumulation of VWF, myeloperoxidase (MPO; a marker of neutrophils), and H3Cit (a marker of NETs) in synovial tissue of the joints was highly reduced as determined by blotting (Figure 2H). To evaluate whether VWF and ADAMTS-13 could be associated with the difference in incidence of arthritis between DBA/1 and C57BL/6 mice, we measured plasma VWF and ADAMTS-13 levels by blotting. Plasma from DBA/1 mice presented significantly higher (approximately 2-fold) level of VWF. ADAMTS-13 was low in both genotypes (Figure 2I). Our results indicate that higher levels of VWF in DBA/1 mice may contribute to the susceptibility to arthritis.

3.3 | The effects of recombinant human ADAMTS-13 on CIA model in DBA/1 J mice after the development of arthritis

Finally, we evaluated the effects of rhADAMTS-13 on mice with arthritis using the DBA/1 J strain in CIA model with LPS to synchronize the incidence of arthritis. We allocated mice into two groups based on arthritis severity to make corresponding severity pairs on day 27, and start consecutive 7-day injections of vehicle or rhADAMTS-13 (Figure 3A). This experiment was done to evaluate the effectiveness of rhADAMTS-13 as a therapeutic strategy. Arthritis severity increased in vehicle-treated mice compared to rhADAMTS-13-treated mice (Figure 3B). There were no differences in serum IL-6 and anti-type II collagen antibody levels when comparing vehicle-treated mice with rhADAMTS-13-treated corresponding-severity mice (Figure 3C). Micro-CT revealed no differences in the corresponding-severity pairs (Figure 3D). Both vehicle- and rhADAMTS-13-treated mice had infiltration of the synovial tissue in the joint space and immunofluorescent staining showed lesions with H3Cit, Ly6G, and DNA in both groups (Figure 3E). However, the histological inflammation scores and synovial hyperplasia scores in rhADAMTS-13-treated mice were significantly lower than those in vehicle-treated mice (Figure 3E and F). The protein expression in synovial tissue revealed rhADAMTS-13-treated mice had reduced levels of VWF and H3Cit compared to corresponding vehicle-treated mice while MPO expression showed no difference in the two groups as revealed by densitometry (Figure 3G).

Our preclinical study has shown the role of *Adamts13* and rhADAMTS-13 in murine arthritis models using three different conditions: the augmentation of arthritis by *Adamts13* deficiency; reduction of VWF and NETs in joints due to rhADAMTS-13 administration, resulting in a decreased incidence and severity of arthritis; and implementation of rhADAMTS-13 in mice with established arthritis, suggesting the usefulness of rhADAMTS-13 in human clinical settings of RA.

We suggest two non–mutually exclusive mechanisms of how ADAMTS-13 reduces the incidence and severity of arthritis. First, ADAMTS-13 can cleave VWF and inhibit the neutrophil recruitment and infiltration to synovial tissue and subsequent NET formation. Second, platelet recruitment by VWF is reduced by ADAMTS-13, and subsequent activated platelets and microparticles are also reduced, leading to less inflammation and local thrombin generation.

The mechanism of the anti-inflammatory effect of ADAMTS-13 on arthritis is still a significant issue. The presence of the anti-CII antibody correlates with the development of arthritis.[9] However, rhADAMTS-13-treated mice presented comparable levels of anti-CII IgG to vehicle-treated mice. This suggests that rhADAMTS-13 affects arthritis through other factors than anti-CII IgG. To explain the effect of ADAMTS-13 on arthritis, we propose the main focus should be around neutrophil infiltration and NETs as the connecting factor between inflammation and the VWF–ADAMTS-13 axis. VWF binds to leukocytes via P-selectin glycoprotein ligand 1 and beta 2 integrins.[11] In addition, NETs have been known not only to bind to VWF, but also to NETs themselves to provide a scaffold to recruit platelets.[12] Based on our results showing reduced NETs (marked by H3Cit) and VWF in the synovial tissue of rhADAMTS-13-treated mice, it is possible that exogenous ADAMTS-13 causes a reduction in neutrophil recruitment and the attachment of VWF to NETs, thus reducing local NET retention. The same mechanism has been shown in skin allograft survival using rhADAMTS-13.[13] Additionally, because NETs perpetuate pathogenic mechanisms and promote immune responses in the joint as well as the periphery in RA,[14] rhADAMTS-13 may lead to the alleviation of arthritis by reducing NET retention. This speculation is consistent with the previous report that ADAMTS-13 reduces VWF-mediated acute inflammation of cerebral ischemia in mice with the decrease of IL-6 and tumor necrosis factor alpha.[15]

Reduced platelet activation due to the reduction of VWF by rhADAMTS-13 may also lead to reduced procoagulant state and inflammation. The inflammation caused by platelets and their activation-induced microparticles are reported in RA.[16] Activated platelets and their microparticles facilitate cellular crosstalk leading to RA by conveying signaling molecules and receptors to the synovium and circulation.[17] In colitis, another inflammatory disease, ADAMTS-13 deficiency worsened and propagated inflammation—most likely through increased platelet–leukocyte recruitment by VWF.[5] Although the source of the VWF is most likely endothelium due to endothelial dysfunction in RA, platelet contribution cannot be excluded. We did not detect platelet markers (CD41 and CD42) by immunostaining and blotting in the synovial tissue.

The CIA model on the DBA/1 background is one of the most studied autoimmune models of RA; however, the CIA model on the C57BL/6 background produced less consistent arthritis changes than those seen on the DBA/1 background. Therefore, our results that C57BL/6 mice, with endogenous ADAMTS-13, exhibit protection from arthritis may not be fully extrapolatable to the CIA model employing DBA/1 mice.

In conclusion, the present study demonstrates the inhibitory role of *Adamts13* in murine arthritis and the effectiveness of rhADAMTS-13 treatment. The effects of rhADAMTS-13 on VWF and NET clearance from the joints is likely the mechanism for alleviated arthritis as indicated by less proliferation of synovial tissue and reduced joint erosions. Therefore, our study suggests that rhADAMTS-13's targeting of inflammation has the potential to treat patients with inflammatory arthritis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by a grant from National Heart, Lung, and Blood Institute of the National Institutes of Health (grant R35 HL135765) to DDW, a grant from Takeda Pharmaceutical Company Ltd to DDW, Grant-in-Aid for JSPS Fellows (to ShF), and The Uehara Memorial Foundation (to ShF). We thank Dr. Bruce Ewenstein for his critical reading of this manuscript and Ms. Kristen Douthit for language editing and proofreading.

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Essentials

- Patients with rheumatoid arthritis have a prothrombotic state leading to frequent thrombotic events.
- This study evaluated the role of von Willebrand factor (VWF) and A Disintegrin And Metalloprotease ThromboSpondin type 1 motif, member 13 (ADAMTS-13) using a mouse inflammatory arthritis model.
- Genetic ADAMTS-13 deficiency in mice exacerbated the development of bone erosion and arthritis, while recombinant ADAMTS-13 showed a protective role in the development and progression of inflammatory arthritis.
- Recombinant ADAMTS-13 reduced neutrophil extracellular traps and VWF retention in mouse synovial tissue.

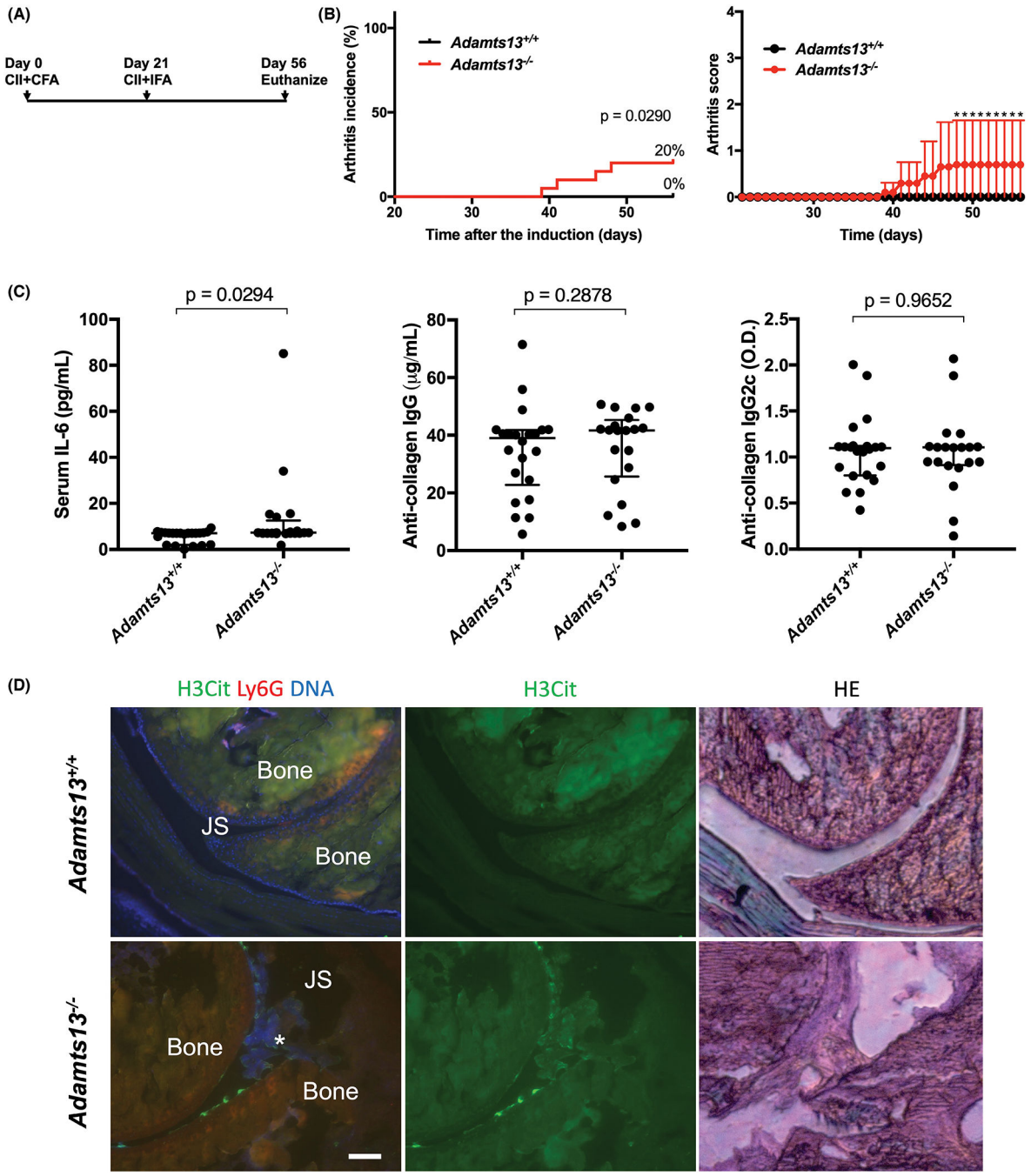

FIGURE 1.

Adamts13-deficient mice develop arthritis while wild-type mice do not. A, Injection schedule. B, Arthritis incidence (left) and severity (right) of $Adamts13^{-/-}$ ($n = 20$) and littermate $Adamts13^{+/+}$ mice ($n = 22$). For arthritis severity, each line shows a mean with a 95% confidence interval. The daily severity scores were used for Wilcoxon's rank-sum test. *$p < .05$. C, serum IL-6, serum anti-collagen IgG antibody and IgG2c antibody on day 56. D, Representative images of immunostainings of Ly6G and citrullinated H3 (H3Cit) and hematoxylin–eosin (HE) stains using ankle joints (JS: joint space, *: synovial tissue, scale

bar = 100 μm). Abbreviations: CII, type II collagen; CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; IL, interleukin.

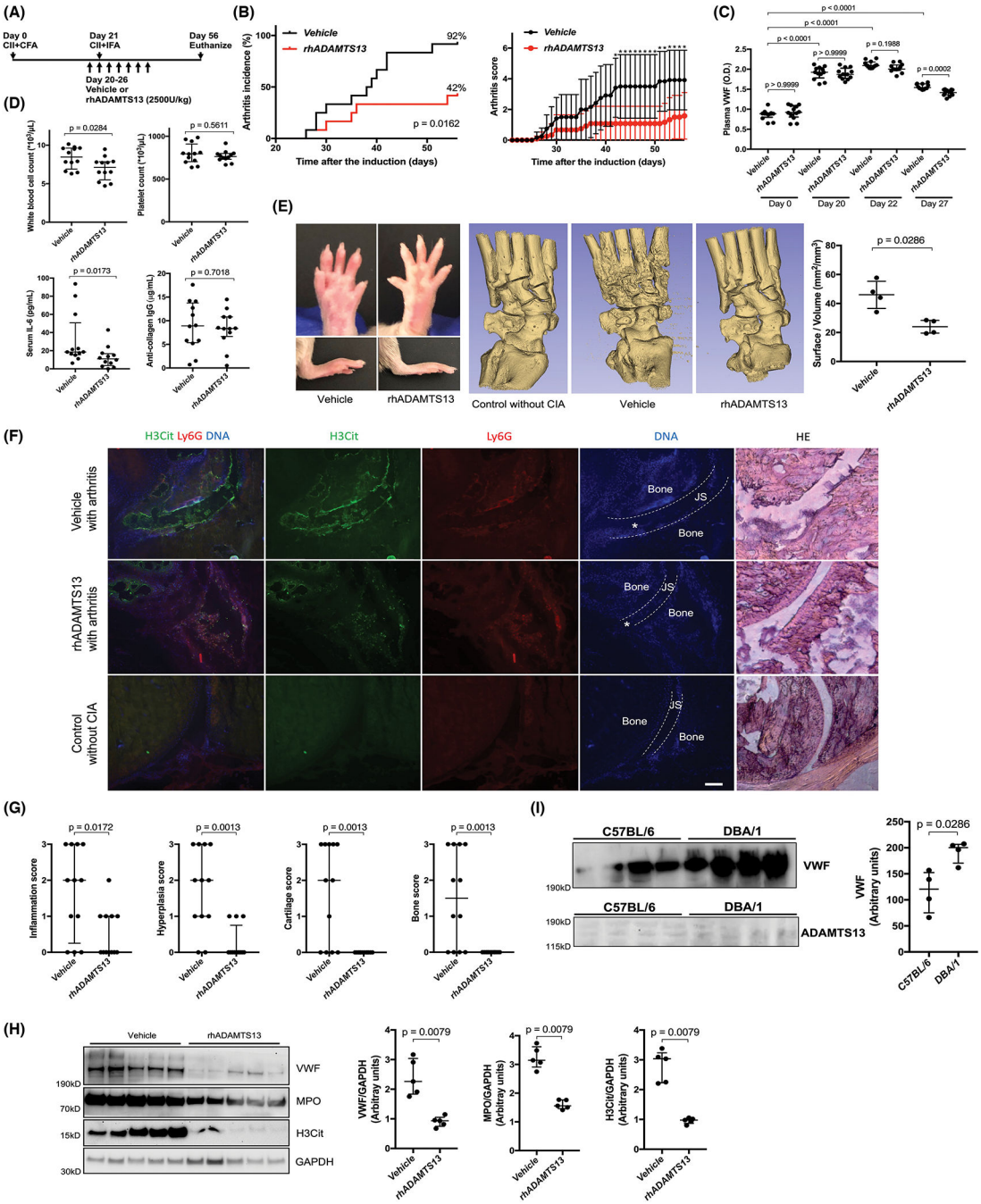

FIGURE 2.

Arthritis alleviation by recombinant human A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif, member 13 (rhADAMTS-13) administered before the development of arthritis. A, Injection schedule. B, Arthritis incidence (left) and severity (right) of vehicle-treated mice and rhADAMTS-13 (2500 U/kg)-treated mice on DBA/1 J strain (n = 12:12). For arthritis severity, each line shows a mean with a 95% confidence interval. The daily severity scores were used for Wilcoxon's rank-sum test. *p <.05. C, The changes in plasma von Willebrand factor (VWF). D, White blood cell and platelet

count on day 22 (upper). Serum interleukin (IL)-6 and serum anti-collagen IgG antibody on day 56 (lower). E, Representative images of paws and micro-computed tomography of joints (left) and quantification of eroded surface of joints (right). F, Immunostaining of Ly6G and citrullinated H3 (H3Cit) and hematoxylin–eosin (HE) stains (JS: joint space, *: synovial tissue, scale bar = 100 μm) (left) and HE stains (right) of ankle joints. G, Quantification of inflammatory cell infiltration, synovial hyperplasia, destruction of articular cartilage, and destruction of bone of ankle joints. H, Blot of synovial tissue (n = 5:5) on VWF, myeloperoxidase (MPO), H3Cit, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; left) and quantification by densitometry (right, relative expression to GAPDH). I, Blot of plasma VWF (upper) and ADAMTS-13 (lower) from wild-type DBA/1 mice and C57BL/6 mice (no induction of arthritis).

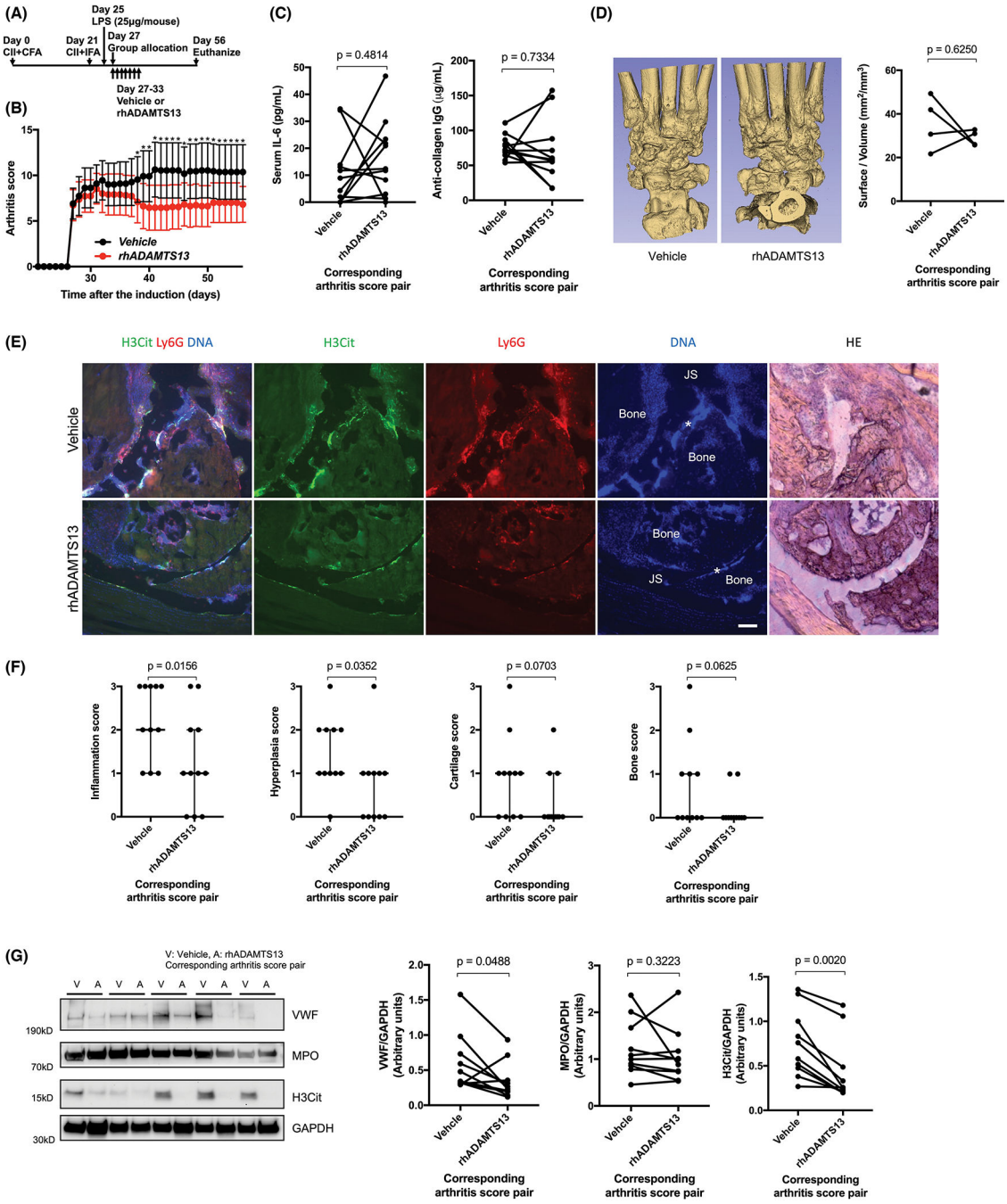

FIGURE 3.

Arthritis alleviation by recombinant human A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif, member 13 (rhADAMTS-13) after the development of arthritis. A, Injection schedule. LPS, lipopolysaccharide. B, Arthritis severity of mice treated with vehicle and treated with rhADAMTS-13 (2500 U/kg) on DBA/1 J strain after incidence of arthritis (n = 11:11). Each line shows a mean with a 95% confidence interval. The daily severity scores were used for Wilcoxon's signed-rank test for corresponding arthritis score pairs. *p <.05. C, Comparison of interleukin (IL)-6 and serum anti-collagen IgG

antibody on day 56 between mice with the corresponding severity determined on day 27. The two points linked by lines indicate two unique mice with equivalent arthritis severity scores on day 27. D, Micro-computed tomography of joints (left) and quantification of the eroded surface of joints with the corresponding severity pair determined on day 27. The two points linked by lines indicate two unique mice with equivalent arthritis severity scores on day 27 (right). E, Hematoxylin–eosin (HE) stain and immunostaining of Ly6G and citrullinated H3 (H3Cit; JS: joint space, *: synovial tissue, scale bar = 100 μm; left) and quantification of inflammation in HE stains (right) using ankle joints. F, Quantification of inflammatory cell infiltration, synovial hyperplasia, destruction of articular cartilage, and destruction of bone of ankle joints with the corresponding severity pair determined on day 27. G, Representative blot of synovial tissue (n = 5:5 of total n = 10:10) of von Willebrand factor (VWF), myeloperoxidase (MPO), H3Cit, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) comparing mice with the corresponding severity determined on day 27 (left) and quantification by densitometry (right, n = 10:10, relative expression to GAPDH). The two points linked by lines indicate two unique mice with equivalent arthritis severity scores on day 27.