

Enhancement of Tissue Factor Expression in Monocyte-Derived Dendritic Cells by Pentraxin 3 and Its Modulation by C1 Esterase Inhibitor

Shogo Kasuda^a Yoshihiko Sakurai^b Kohei Tatsumi^c Tomohiro Takeda^d
Risa Kudo^a Katsuya Yuui^a Katsuhiko Hatake^a

^aDepartment of Legal Medicine, Nara Medical University, Kashihara, Japan; ^bDepartment of Pediatrics, Matsubara Tokushukai Hospital, Matsubara, Japan; ^cDepartment of Physiology and Regenerative Medicine, Kindai University Faculty of Medicine, Osaka-Sayama, Japan; ^dDepartment of Clinical Laboratory Science, Kansai University of Health Sciences, Kumatori, Japan

Keywords

Complement inhibitors · Monocyte-derived dendritic cells · Pentraxin 3 · Shear · Tissue factor

Abstract

Background: We have previously shown that human monocyte-derived dendritic cells (moDCs) may participate in immune system-mediated hypercoagulable state through enhanced tissue factor (TF) expression and that the complement system may be involved in this process. **Objectives:** The aim of this study was to explore the role of pentraxin 3 (PTX3) and the complement system in enhanced TF expression in moDCs. **Methods:** moDCs were generated from isolated human monocytes. PTX3 levels in whole human blood supplemented with moDCs were determined after lipopolysaccharide (LPS) stimulation. PTX3 release by the generated moDCs upon LPS stimulation was also assessed. The effect of PTX3 on whole blood coagulation was investigated using thromboelastometric analysis. TF expression in stationary moDCs treated with LPS and/or PTX3 was determined by measuring TF activity. The effect of complement inhibitors on TF activity in moDCs treated with LPS and/or PTX3 under low-shear conditions was evaluated. **Results:** PTX3 levels were higher in whole blood supplemented with moDCs than

in the presence of monocytes and were further elevated by LPS stimulation. PTX3 release from generated moDCs was also increased by LPS stimulation. PTX3 reduced whole blood coagulation time in a dose-dependent manner. However, PTX3 did not increase TF expression in stationary moDCs. Under low-shear conditions, PTX3 increased TF expression in moDCs. C1 esterase inhibitor (C1-inh) suppressed this effect. **Conclusions:** PTX3 might have a thrombophilic activity and enhance TF expression in moDCs under low-shear conditions. Furthermore, suppression of moDC-associated hypercoagulability by C1-inh might be partly ascribed to its inhibitory effect on PTX3.

© 2019 S. Karger AG, Basel

Introduction

Monocytes play a major role in thrombus formation under inflammatory conditions, i.e., immunothrombosis [1]. Immunothrombosis facilitates the recognition, containment, and destruction of pathogens [1, 2]. However, exacerbation of immunothrombosis beyond control can be detrimental, leading to organ failure or even mortality through

Edited by: H.-U. Simon, Bern.

disseminated intravascular coagulation and atherothrombosis [3–5]. Therefore, regulating immunothrombosis would be critical for the treatment of such conditions.

Monocyte-derived dendritic cells (moDCs), differentiated from monocytes under inflammatory conditions, might also be involved in immunothrombosis. Indeed, in a previous study [6], we showed that whole blood coagulation time, as assessed by thromboelastometric analysis, is significantly lower in whole blood supplemented with moDCs than in whole blood alone or in whole blood supplemented with monocytes. These findings suggested that moDCs might play a more important role than monocytes in immunothrombosis. We also found that the moDC-associated hypercoagulable state is linked to enhanced expression of tissue factor (TF). TF is now known to be involved in the process of angiogenesis [7, 8] as well as the major trigger for the clotting cascade; its pathological expression may drive thrombosis [9]. Furthermore, we showed that modulation of the complement system by C1 esterase inhibitor (C1-inh) leads to the inhibition of TF expression in moDCs, suggesting that moDC activation is associated with the complement system. However, the detailed mechanism of TF expression enhancement in moDCs and the inhibitory activity of C1-inh on TF expression in moDCs remain to be elucidated.

Pentraxin 3 (PTX3) is a key component of the humoral arm of the innate immune system [10]. Previous studies demonstrated that PTX3 upregulates TF expression in monocytes and is involved in the stimulation of TF expression in monocytes by lipopolysaccharide (LPS) [11, 12]. In addition, the inhibitory activity of C1-inh on LPS-induced TF expression in monocytes is partly ascribed to the suppression of PTX3 generation [12]. Nevertheless, subsequent studies failed to confirm these findings in vivo and rather showed a cardiovascular protective property of PTX3 [13, 14]. Recently, however, PTX3 has attracted attention owing to its multifaceted effects on the cardiovascular system [15].

In the current study, we aimed to elucidate the involvement of PTX3 in TF expression upregulation in moDCs and the prothrombotic property of PTX3. We also aimed to explore the involvement of C1 in PTX3-associated TF expression in moDCs.

Materials and Methods

Reagents

Purified human C1-inh (Berinert®) was purchased from CSL Behring (Tokyo, Japan). C3-binding cyclic synthetic peptide (C3-inh, compstatin) was obtained from Bioscience (Ellisville, MO,

USA). The doses of C1-inh and C3-inh were determined according to a previously described method [12, 16]. Octyl-β-D-glucopyranoside and LPS (*E. coli* O11:B4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). T-PER tissue protein extract reagent, protease inhibitor cocktails, and phosphatase inhibitors were from Thermo Fisher Scientific (Rockford, IL, USA). Pyrogen-free recombinant human PTX3 was from R&D Systems (Minneapolis, MN, USA). All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless noted otherwise.

Isolation of Monocytes and Generation of moDCs

moDCs were obtained from the blood of healthy volunteers comprising 3 males and 3 females 30–50 years of age who had provided written informed consent and then taken no medication for 2 weeks before blood sampling. Briefly, peripheral blood mononuclear cells were separated from citrated whole blood using Lymphoprep (Axis-Shield, Oslo, Norway). Biomagnetic negative depletion of classical monocytes from peripheral blood mononuclear cells was performed using a Dynabeads Untouched Human Monocytes kit (Invitrogen, Carlsbad, CA, USA). Isolated CD14-positive CD16-negative classical monocytes were differentiated into dendritic cells, and the purity of the generated moDCs was confirmed (>94%) by flow cytometry using mouse antihuman CD11b, CD11c, CD83, CD85, DC-SIGN antibodies, as described previously [6]. To eliminate the hemostatic effects of the individual differences between subjects, comparisons between monocytes and moDCs in the following experiments were made using the same donor-derived monocytes and moDCs. Isolated monocytes and moDCs were resuspended in RPMI and 10% fetal bovine serum.

PTX3 Levels

To evaluate the effects of the interactions among moDCs, peripheral blood cells, and plasma components on PTX3 release, 15 μL of moDCs or monocytes (final concentration [f.c.] 30 cells/μL) were mixed with 475 μL of whole blood in 1.5-mL microtubes and incubated for 2 h at 37 °C with 10 μL of LPS (f.c. 1 μg/mL) or vehicle (phosphate-buffered saline [PBS]). After centrifugation, plasma PTX3 levels were determined by human-specific ELISA kits (R&D Systems) according to the manufacturer's instructions.

Next, to assess PTX3 release from moDCs, the generated moDCs (2.5×10^5 cells) were seeded in 24-well culture plates (500 μL/well) and mixed with LPS (f.c. 1 μg/mL) or vehicle (PBS). After incubation at 37 °C for 24 h and centrifugation, PTX3 levels in the supernatant were determined as described above.

Rotational Thromboelastometry

The effects of PTX3 on whole blood coagulation were investigated using rotational thromboelastometry (ROTEM), an advanced type of conventional thromboelastography which measures the global viscoelastic properties of whole blood clot formation. Briefly, in ROTEM, a plastic pin rotates in the center of a fixed small plastic cup with whole blood, which produces low shear conditions. Changes in torque between the pin and the cup because of fibrin strands from blood clots are detected optically and calculated to the numerical parameters. The clotting time (CT) with clot formation time (CFT), indicative of the whole blood coagulation time, was adopted as an indicator of hypercoagulability, as previously described [6] (online suppl. Fig. 1; see www.karger.com/doi/10.1159/000496744 for all online suppl.

material). For these experiments, 20 μL of various concentrations of PTX3 or vehicle (PBS) were added to 380 μL of normal blood. After incubation at 37 °C for 2 h, an ROTEM assay was initiated by the addition of 20 μL of 0.2 M CaCl_2 in the nonactivating thromboelastometry mode.

TF Activity Assay

The TF activity of moDCs was determined after PTX3 treatment. PTX3 (f.c. 5 $\mu\text{g}/\text{mL}$) or vehicle (PBS) was added to a moDC or monocyte suspension, with or without LPS (f.c. 1 $\mu\text{g}/\text{mL}$). After incubation at 37 °C for 6 h, the samples were washed twice with PBS and suspended in 15 mM octyl- β -D-glucopyranoside at 37 °C for 15 min. TF activities in cell lysates were assessed using a Tissue Factor Chromogenic Activity kit (Assaypro, St. Charles, MO, USA) according to the manufacturer's instructions. The raw data of TF activity were corrected for the amount of total protein determined by the bicinchoninic acid assay (Thermo Fisher Scientific). The results are expressed as a ratio of values of treated cells to those of control cells.

Shear Stress Experiment

To elucidate the role of shear stress in PTX3-induced TF expression in moDCs, moDCs were exposed to low-shear force using an ROTEM analyzer. For the experiment, 20 μL of PTX3 (f.c. 5 $\mu\text{g}/\text{mL}$) or vehicle (PBS) were added to the moDC suspension (1×10^5 cells in 280 μL) in the presence or absence of C1-inh (f.c. 2.5 $\mu\text{g}/\text{mL}$) or C3-inh (f.c. 31 $\mu\text{g}/\text{mL}$). Cells were incubated under stationary conditions at 37 °C for 4 h and exposed to low-shear conditions for 2 h using an ROTEM analyzer. moDCs treated with PTX3 alone and incubated under stationary conditions at 37 °C for 6 h were used as a control. The cells were harvested by centrifugation and TF activities were evaluated as described above.

Statistical Analysis

Statistical analyses were performed using SPSS software v.19 (IBM SPSS Statistics, Chicago, IL, USA). Data were analyzed using the Student *t* test or, if more than two experimental groups were compared, one-way ANOVA followed by the Tukey-Kramer post-test. Data are expressed as mean $\pm$ standard error of the mean, and *p* values <0.05 were considered significant.

Results

LPS Affected PTX3 Release from moDCs in Whole Blood

Plasma PTX3 levels in whole blood after 2-h incubation at 37 °C are shown in Figure 1a. The addition of monocytes to whole blood did not alter the plasma PTX3 levels. Although LPS stimulation of whole blood increased PTX3 levels, no difference in PTX3 levels was observed in LPS-stimulated whole blood upon monocyte supplementation. By contrast, moDC supplementation of whole blood that had not been stimulated by LPS significantly increased plasma PTX3 levels in comparison to those in unsupplemented LPS-stimulated whole blood

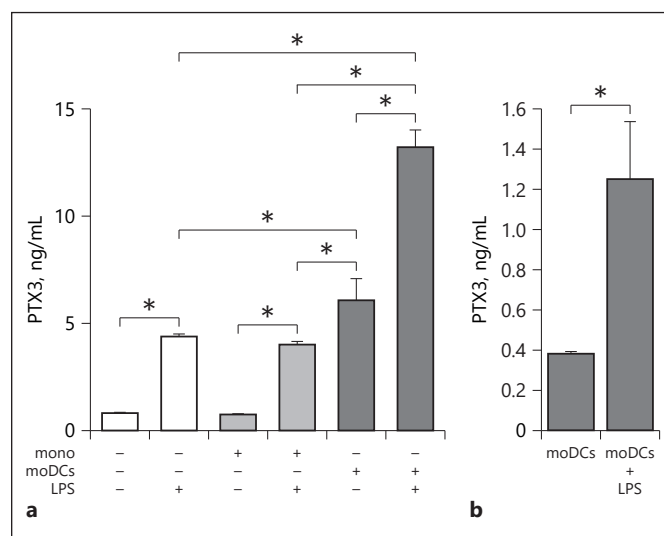


Fig. 1. PTX3 release from whole blood and moDCs. **a** PTX3 release from whole blood in the presence or absence of monocytes or moDCs, with or without LPS stimulation. **b** PTX3 release from moDCs alone, with or without LPS stimulation. Specific PTX3 release from moDCs was significantly enhanced by LPS. Error bars represent the SEM from five independent measurements. * *p* < 0.05. LPS, lipopolysaccharide; moDCs, monocyte-derived dendritic cells; mono, monocytes; PTX3, pentraxin 3; SEM, standard error of the mean.

regardless of the presence of monocytes. LPS stimulation of whole blood supplemented with moDCs further increased plasma PTX3 levels.

LPS Stimulation Increased PTX3 Release from the Generated moDCs

To verify the source of PTX3 in whole blood supplemented with moDCs, PTX3 levels in the supernatant of generated moDCs were determined. After LPS stimulation, PTX3 levels increased by approximately three times in cell suspension supernatant (Fig. 1b).

PTX3 Enhanced Whole Blood Coagulation

Thromboelastometric analysis using an ROTEM analyzer revealed that the addition of PTX3 to whole blood (in the absence of moDC supplementation) significantly shortened the CT+CFT value in a dose-dependent manner (Fig. 2; online suppl. Fig. 2). Reduction of the CT+CFT value was already observed in the presence of 0.25 $\mu\text{g}/\text{mL}$ of PTX3, although it was not statistically significant. A significant reduction of the CT+CFT value was evident in the presence of 1.0 $\mu\text{g}/\text{mL}$ (and more) of PTX3.

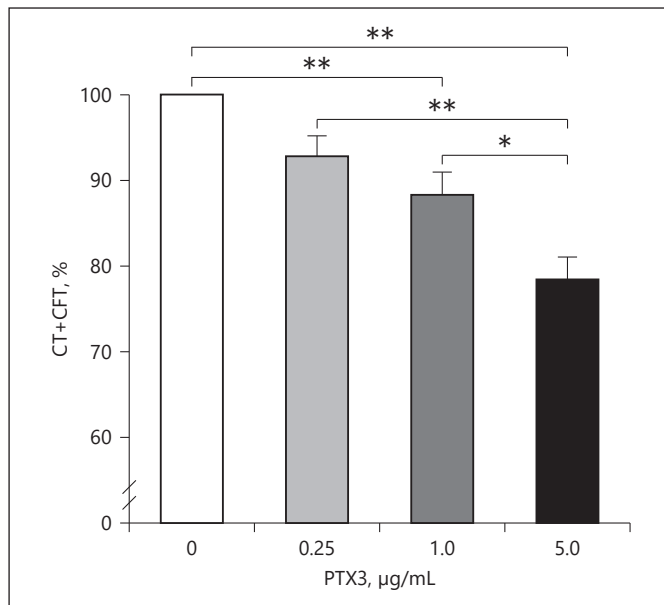


Fig. 2. The effect of PTX3 on whole blood coagulation. The thromboelastometric variable CT+CFT was assessed in the presence of PTX3. The CT+CFT value of control samples without PTX3 was set as 100%. The values in the presence of PTX3 are expressed relative (%) to values for the control samples. PTX3 reduced the CT+CFT value in a dose-dependent manner. Error bars represent the SEM from five independent measurements. * $p < 0.05$, ** $p < 0.01$. CFT, clot formation time; CT, clotting time; PTX3, pentraxin 3; SEM, standard error of the mean.

PTX3-Associated TF Expression in moDCs Required Shear Force

Under stationary conditions, PTX3 alone did not increase TF activity in moDCs. In addition, the LPS-induced increase in TF expression was not further enhanced in the presence of PTX3 under these conditions (Fig. 3a). Based on our hypothesis that shear force is required for PTX3-associated TF expression in moDCs, moDCs were incubated, with or without PTX3, under low-shear conditions. Although TF activity was not altered in the absence of PTX3, the activity was significantly elevated in the presence of PTX3 under low-shear conditions (Fig. 3b). Of note, C1-inh suppressed the PTX3-enhanced increase of TF activity under low-shear conditions, while C3-inh did not (Fig. 3b).

Discussion

In the present study, we showed that PTX3 levels in whole blood were significantly elevated after LPS stimulation. More importantly, PTX3 levels in whole blood sup-

plemented with moDCs were significantly higher than those in whole blood with monocytes and those in whole blood alone. This indicated that moDCs more potently generated PTX3 than monocytes. The PTX3 levels in whole blood supplemented with moDCs were further elevated by LPS stimulation. This suggested that, similar to monocyte PTX3 production, LPS probably stimulated PTX3 generation by moDCs. Accordingly, we confirmed that PTX3 release from the generated moDCs was significantly enhanced by LPS stimulation (Fig. 1b). The effect of LPS on PTX3 generation in moDCs was significant after a 2-h incubation in the whole blood environment (Fig. 1a). However, the effect of LPS on PTX3 generation in moDCs alone, i.e., in the absence of whole blood, was not observed until 24 h of incubation with much less PTX3 generation compared with that in the whole blood environment despite the higher moDC concentration (Fig. 1b). These results suggest that the whole blood environment will facilitate PTX3-generating cells such as moDCs to enhance generation of PTX3.

Long pentraxin, PTX3, is generated under inflammatory conditions by a wide variety of human cells [17, 18]. In particular, human moDCs produce large amounts of PTX3 in response to pathogen-associated molecular patterns, including LPS [19, 20]. The current study further supports these findings, which raised a question about the role of PTX3 produced in moDCs in whole blood coagulation. Differently from the short pentraxin C-reactive protein that is produced only in the liver, PTX3 is produced systemically and has attracted attention as a sensitive biomarker of infection and vascular inflammation [21, 22]. Whether PTX3 is proinflammatory or anti-inflammatory [23], procoagulant or anticoagulant, and cardiovascular protective or threatening remains controversial. Thromboelastometric analysis performed in the current study revealed that even in the absence of moDC supplementation, PTX3 could elicit a hypercoagulable state of whole blood (Fig. 2). Previous studies demonstrated that PTX3 upregulates TF expression in human monocytes [11] and that PTX3 is involved in LPS-enhanced TF expression in monocytes [12]. These findings suggest that elevated TF production by monocytes, which constitute a small fraction of whole blood, induced by PTX3 might contribute to PTX3-induced hypercoagulability of whole blood in the experimental system used. We therefore speculated that moDCs might accelerate coagulation by upregulating TF expression via PTX3, similarly to monocytes. However, contrary to the hypothesis, under stationary conditions, the TF activity of moDCs was not altered in the presence of PTX3 (Fig. 3a). Neverthe-

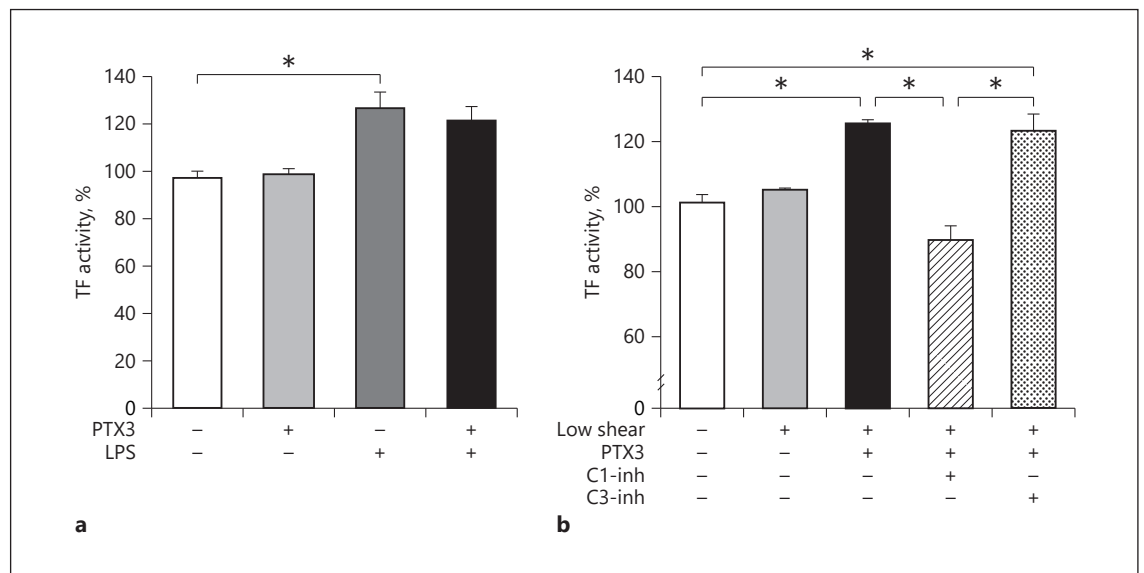


Fig. 3. The effect of PTX3 on TF activity in moDCs. **a** TF activity of stationary moDCs incubated with PTX3. Incubation of stationary moDCs with LPS increased TF activity, but the addition of PTX3 to LPS did not result in further increase. **b** TF activity of moDCs incubated with PTX3 under low-shear conditions. TF activity was enhanced by PTX3. PTX3-associated increase in TF activity

was suppressed by C1-inh, but not by C3-inh. Error bars represent the SEM from five independent measurements. * $p < 0.05$. C1-inh, C1 esterase inhibitor; C3-inh, C3-binding cyclic synthetic peptide; LPS, lipopolysaccharide; moDCs, monocyte-derived dendritic cells; PTX3, pentraxin 3; SEM, standard error of the mean; TF, tissue factor.

less, PTX3-treated moDCs exhibited a significant increase in TF activity under low-shear conditions (Fig. 3b), suggesting that PTX3 participates, at least to some extent, in the increased TF expression in moDCs under inflammatory conditions in vivo. Napoleone et al. [11] reported that PTX3 enhances TF synthesis in monocytes by enhancing the nuclear binding activity of the transacting factor c-Rel/p65 from the nuclear factor κ B family. A similar mechanism might induce TF expression in moDCs, but further studies are required to delineate it.

The observations reported herein also indicated that low-shear forces might be essential for PTX3-mediated augmentation of TF expression in moDCs, while PTX3 is released by moDCs under stationary conditions. Low-shear stress increases inflammation, lipid content, and intercellular permeability and initiates atherosclerotic plaque formation [24–26]. The shear rate produced by the ROTEM analyzer, which facilitated the upregulation of TF synthesis in moDCs, was low (0.1 s^{-1}), resembling a sluggish flow that favors the development of atherosclerosis [27, 28]. The presented results further supported the notion of thrombus formation on atherosclerotic plaques. A number of moDCs reside in atherosclerotic plaques and change into foam cells by taking up LDL [29, 30]. Furthermore, foam cells express PTX3 in atherosclerotic

lesions [31]. When a plaque ruptures, moDCs with a prothrombotic profile are exposed to blood streaming. Subsequent pathogenic thrombosis might easily occur at the atherosclerotic plaque rupture site. It is plausible that podosomes participate in moDC activation by shear stress. Podosomes are produced by cells from the monocytic lineage, including moDCs, and are involved in the contact between the cell and the extracellular matrix [32]. Tension applied to moDCs by the blood flow stretches mechanosensitive proteins of the podosome, which might lead to intracellular signaling [33]. Further studies are warranted to elucidate the underlying mechanisms.

The complement and coagulation systems intimately and mutually affect one another to provide both positive and negative feedbacks for hemostasis and inflammation [34]. C1-inh hampers all (three) complement activation pathways by irreversibly binding to, and inactivating, proteases C1r and C1s. In addition, since C1-inh is the most potent physiological inhibitor of activated coagulation factors XI and XII as well as kallikrein, the contact activation pathway (intrinsic pathway) is blocked by C1-inh [35]. This is further supported by a recent report that relied on thromboelastometry to show that C1-inh suppresses whole blood coagulation [16]. This suggests that C1-inh deficiency, which is known as type I and II he-

editary angioedema (C1-inh-HAE), may increase the risk of thrombosis. Although C1-inh-HAE patients rarely suffer from thrombotic events, D-dimer levels are elevated in association with C1-inh-HAE attacks in these patients [36]. Furthermore, deterioration of the biosynthesis of antithrombin and TF pathway inhibitor during aging alters the characteristic symptoms of the disease from the occurrence of localized edema to the onset of a hypercoagulable state of blood [37]. Since the levels of antithrombin and TF pathway inhibitor decrease in the presence of inflammation [38, 39], a thrombotic event could occur even in C1-inh-HAE patients who are not elderly. Furthermore, in C1-inh-HAE, neutrophil activation occurs under inflammatory conditions. It is driven by proinflammatory cytokines, such as tumor necrosis factor α and interleukin 8, leading to myeloperoxidase release and the formation of neutrophil extracellular traps that promote thrombosis [40]. Collectively, these findings indicate the existence of hypercoagulable state in C1-inh-HAE patients under inflammatory conditions and support the anticoagulant properties of C1-inh.

Landsem et al. [12] reported that the inhibition of LPS-induced TF expression in monocytes by C1-inh may be partly ascribed to the suppression of PTX3 generation. Previously, we showed that the inhibition of moDC-induced hypercoagulation by C1-inh likely results from the suppression of TF expression. In the present study, we showed that C1-inh suppressed the PTX3-enhanced TF expression in moDCs under low-shear conditions. Therefore, the inhibitory activity of C1-inh on TF expression induced by LPS in moDCs might be associated with the inhibition of PTX3 release and inhibition of the contact and/or intrinsic coagulation of plasma.

Notably, the PTX3 concentration used in the current study (5 $\mu\text{g/mL}$) was higher than the nominal PTX3 levels in circulating serum [41]. However, in the experiments investigating the effects of PTX3 on whole blood coagulation, 0.25 $\mu\text{g/mL}$ of PTX3 indeed exerted some procoagulant activity, although it was not statistically significant (the activity increase became significant when 1.0 $\mu\text{g/mL}$ PTX3 was used). However, considering that PTX3 levels in the circulating blood increase to 1.5 $\mu\text{g/mL}$ under inflammatory conditions [42], the maximum levels at an inflammatory site might be several times higher than those in the circulating blood, and possibly beyond the concentration used in the experiments. We therefore believe that the PTX3 concentrations used in the current study are clinically relevant.

In conclusion, we showed in the present study that PTX3 induces hypercoagulability of whole blood and TF

synthesis in moDCs. moDC-induced hypercoagulation may be accelerated partially by PTX3 generated by moDCs themselves. moDCs, which are induced from classical monocytes under inflammatory conditions and accumulate at inflammatory sites in vivo (i.e., under shear stress conditions), may contribute to the formation of pathological immunothrombus by TF expression and PTX3 release. The present findings shed light on the role of moDCs in the mechanism of immunothrombosis and atherothrombosis. Furthermore, C1-inh might suppress a series of events in the inflammation-coagulation axis associated with moDCs and may be beneficial for treating pathological immunothrombosis.

Acknowledgment

The authors thank Emeritus Professor Yoshihiro Fujimura (Nara Medical University) for critical reading of the manuscript.

Statement of Ethics

Written informed consent was obtained from all participants included in the study. All procedures involving human participants were in accordance with the ethical standards of the Nara Medical University Ethics Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by grants-in-aid from the Cardiovascular Research Fund, Tokyo, Japan (to S. Kasuda). The sponsor did not play any role in the preparation of data or the manuscript.

Author Contributions

S. Kasuda designed the study, performed laboratory studies, analyzed the data, and drafted the manuscript. Y. Sakurai designed the study, interpreted the data, and edited the manuscript. K. Tatum analyzed the data and edited the manuscript. T. Takeda performed the flow cytometry analysis, analyzed the data, and edited the manuscript. R. Kudo and K. Yuui performed laboratory studies, analyzed the data, and assisted with manuscript preparation. K. Hatake assisted with the study design, supervised laboratory work, and provided intellectual contribution to the manuscript.

References

- Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol*. 2013 Jan;13(1):34–45.
- Alcock J, Brainard AH. Hemostatic containment – an evolutionary hypothesis of injury by innate immune cells. *Med Hypotheses*. 2008 Dec;71(6):960–8.
- Ito T. PAMPs and DAMPs as triggers for DIC. *J Intensive Care*. 2014 Dec;2(1):67.
- Pfeiler S, Massberg S, Engelmann B. Biological basis and pathological relevance of microvascular thrombosis. *Thromb Res*. 2014 May;133 Suppl 1:S35–7.
- Gaertner F, Massberg S. Blood coagulation in immunothrombosis – at the frontline of intravascular immunity. *Semin Immunol*. 2016 Dec;28(6):561–9.
- Kasuda S, Sakurai Y, Tatsumi K, Takeda T, Kudo R, Yuui K, et al. Experimental hypercoagulable state induced by tissue factor expression in monocyte-derived dendritic cells and its modulation by C1 inhibitor. *J Thromb Thrombolysis*. 2018 Aug;46(2):219–26.
- Carmeliet P, Mackman N, Moons L, Luther T, Gressens P, Van Vlaenderen I, et al. Role of tissue factor in embryonic blood vessel development. *Nature*. 1996 Sep;383(6595):73–5.
- Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol*. 2004 Jun;24(6):1015–22.
- Owens AP 3rd, Mackman N. Tissue factor and thrombosis: the clot starts here. *Thromb Haemost*. 2010 Sep;104(3):432–9.
- Jaillon S, Bonavita E, Gentile S, Rubino M, Laface I, Garlanda C, et al. The long pentraxin PTX3 as a key component of humoral innate immunity and a candidate diagnostic for inflammatory diseases. *Int Arch Allergy Immunol*. 2014;165(3):165–78.
- Napoleone E, di Santo A, Peri G, Mantovani A, de Gaetano G, Donati MB, et al. The long pentraxin PTX3 up-regulates tissue factor in activated monocytes: another link between inflammation and clotting activation. *J Leukoc Biol*. 2004 Jul;76(1):203–9.
- Landsem A, Nielsen EW, Fure H, Christiansen D, Ludviksen JK, Lambris JD, et al. C1-inhibitor efficiently inhibits *Escherichia coli*-induced tissue factor mRNA up-regulation, monocyte tissue factor expression and coagulation activation in human whole blood. *Clin Exp Immunol*. 2013 Aug;173(2):217–29.
- Norata GD, Garlanda C, Catapano AL. The long pentraxin PTX3: a modulator of the immunoinflammatory response in atherosclerosis and cardiovascular diseases. *Trends Cardiovasc Med*. 2010 Feb;20(2):35–40.
- Bonacina F, Barbieri SS, Cutuli L, Amadio P, Doni A, Sironi M, et al. Vascular pentraxin 3 controls arterial thrombosis by targeting collagen and fibrinogen induced platelets aggregation. *Biochim Biophys Acta*. 2016 Jun;1862(6):1182–90.
- Fornai F, Carrizzo A, Forte M, Ambrosio M, Damato A, Ferrucci M, et al. The inflammatory protein Pentraxin 3 in cardiovascular disease. *Immun Ageing*. 2016 Aug;13(1):25.
- Landsem A, Fure H, Mollnes TE, Nielsen EW, Brekke OL. C1-inhibitor efficiently delays clot development in normal human whole blood and inhibits *Escherichia coli*-induced coagulation measured by thromboelastometry. *Thromb Res*. 2016 Jul;143(Jul):63–70.
- Doni A, Peri G, Chieppa M, Allavena P, Pasqualini F, Vago L, et al. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. *Eur J Immunol*. 2003 Oct;33(10):2886–93.
- Rolph MS, Zimmer S, Bottazzi B, Garlanda C, Mantovani A, Hansson GK. Production of the long pentraxin PTX3 in advanced atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. 2002 May;22(5):e10–4.
- Breviario F, d'Aniello EM, Golay J, Peri G, Bottazzi B, Bairoch A, et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem*. 1992 Nov;267(31):22190–7.
- Maina V, Cotena A, Doni A, Nebuloni M, Pasqualini F, Milner CM, et al. Coregulation in human leukocytes of the long pentraxin PTX3 and TSG-6. *J Leukoc Biol*. 2009 Jul;86(1):123–32.
- Uusitalo-Seppälä R, Huttunen R, Aittoniemi J, Koskinen P, Leino A, Vahlberg T, et al. Pentraxin 3 (PTX3) is associated with severe sepsis and fatal disease in emergency room patients with suspected infection: a prospective cohort study. *PLoS One*. 2013;8(1):e53661.
- Bastrup-Birk S, Skjoedt MO, Munthe-Fog L, Strom JJ, Ma YJ, Garred P. Pentraxin-3 serum levels are associated with disease severity and mortality in patients with systemic inflammatory response syndrome. *PLoS One*. 2013 Sep;8(9):e73119.
- Kunes P, Holubcova Z, Kolackova M, Krejssek J. Pentraxin 3 (PTX3): an endogenous modulator of the inflammatory response. *Mediators Inflamm*. 2012;2012:920517.
- Okano M, Yoshida Y. Junction complexes of endothelial cells in atherosclerosis-prone and atherosclerosis-resistant regions on flow dividers of brachiocephalic bifurcations in the rabbit aorta. *Biorheology*. 1994 Mar–Apr;31(2):155–61.
- Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA*. 1999 Dec;282(21):2035–42.
- Morbiducci U, Kok AM, Kwak BR, Stone PH, Steinman DA, Wentzel JJ. Atherosclerosis at arterial bifurcations: evidence for the role of haemodynamics and geometry. *Thromb Haemost*. 2016 Mar;115(3):484–92.
- Bartal C, Yitzhak A. The role of thromboelastometry and recombinant factor VIIa in trauma. *Curr Opin Anaesthesiol*. 2009 Apr;22(2):281–8.
- Whiting D, DiNardo JA. TEG and ROTEM: technology and clinical applications. *Am J Hematol*. 2014 Feb;89(2):228–32.
- Haka AS, Singh RK, Grosheva I, Hoffner H, Capetillo-Zarate E, Chin HF, et al. Monocyte-Derived Dendritic Cells Upregulate Extracellular Lipoprotein on Maturation, Leading to Foam Cell Formation. *Arterioscler Thromb Vasc Biol*. 2015 Oct;35(10):2092–103.
- Salvatore G, Bernoud-Hubac N, Bissay N, Debarb C, Daira P, Meugnier E, et al. Human monocyte-derived dendritic cells turn into foamy dendritic cells with IL-17A. *J Lipid Res*. 2015 Jun;56(6):1110–22.
- Savchenko A, Imamura M, Ohashi R, Jiang S, Kawasaki T, Hasegawa G, et al. Expression of pentraxin 3 (PTX3) in human atherosclerotic lesions. *J Pathol*. 2008 May;215(1):48–55.
- Block MR, Badowski C, Millon-Fremillon A, Bouvard D, Bouin AP, Faurobert E, et al. Podosome-type adhesions and focal adhesions, so alike yet so different. *Eur J Cell Biol*. 2008 Sep;87(8–9):491–506.
- Linder S, Wiesner C. Feel the force: podosomes in mechanosensing. *Exp Cell Res*. 2016 Apr;343(1):67–72.
- Amara U, Rittirsch D, Flierl M, Bruckner U, Klos A, Gebhard F, et al. Interaction between the coagulation and complement system. *Adv Exp Med Biol*. 2008;632:71–9.
- Davis AE 3rd. Biological effects of C1 inhibitor. *Drug News Perspect*. 2004 Sep;17(7):439–46.
- Reshef A, Zanichelli A, Longhurst H, Relan A, Hack CE. Elevated D-dimers in attacks of hereditary angioedema are not associated with increased thrombotic risk. *Allergy*. 2015 May;70(5):506–13.
- Kodama J, Uchida K, Kushiro H, Murakami N, Yutani C. Hereditary angioneurotic edema and thromboembolic diseases: I. How symptoms of acute attacks change with aging. *Intern Med*. 1998 May;37(5):440–3.
- Maroney SA, Mast AE. Tissue factor pathway inhibitor and bacterial infection. *J Thromb Haemost*. 2011 Jan;9(1):119–21.
- Cosgriff TM, Jahrling PB, Chen JP, Hodgson LA, Lewis RM, Green DE, et al. Studies of the coagulation system in arenaviral hemorrhagic fever: experimental infection of strain 13 guinea pigs with Pichinde virus. *Am J Trop Med Hyg*. 1987 Mar;36(2):416–23.
- Veseli N, Csuka D, Zotter Z, Imreh É, Józsi M, Benedek S, et al. Neutrophil activation during attacks in patients with hereditary angioedema due to C1-inhibitor deficiency. *Orphanet J Rare Dis*. 2015 Dec;10(Dec):156.
- Yamasaki K, Kurimura M, Kasai T, Sagara M, Kodama T, Inoue K. Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations. *Clin Chem Lab Med*. 2009;47(4):471–7.
- Daigo K, Mantovani A, Bottazzi B. The yin-yang of long pentraxin PTX3 in inflammation and immunity. *Immunol Lett*. 2014 Sep;161(1):38–43.