

Role of TF-Triggered Activation of the Coagulation Cascade in the Pathogenesis of Chronic Spontaneous Urticaria

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Abbreviations CSU Chronic spontaneous urticarial · CIU Chronic idiopathic urticarial · TF Tissue factor · PAR Protease-activated receptor · FcεRI High-affinity IgE receptor · HUVEC Human umbilical vein endothelial cells · HMEC Human dermal microvascular endothelial cells · TLR Toll-like receptor · LPS Lipopolysaccharide · IL Interleukin · LPS Lipopolysaccharides · SP Substance P · MRGX2 MAS-related G protein-coupled receptor member X2 · NMU Neuromedin U · MBP Major basic protein · EPO Eosinophil peroxidase · CRP C-reactive protein · PAF Platelet-activating factor

Abstract

Purpose of Review To overview recent understanding of the relationship between the blood coagulation cascade and chronic spontaneous urticaria (CSU).

Recent Findings The relationship between severities of CSU and the increase of coagulation markers, and the effectiveness of anti-coagulants, such as warfarin, suggest a causative role of the blood coagulation in the pathogenesis of CSU. However, mechanisms of the initiation of blood coagulation, and the link between blood coagulation and wheal formation in urticaria, remained unclear. In blood vessels, vascular endothelial cells and eosinophils may express tissue factor (TF), which triggers the activation of extrinsic coagulation cascade. We recently revealed that histamine and TLR agonists synergistically induce TF expression by endothelial cells and produce active forms of coagulation factors, such as Xa and IIa, which may induce plasma extravasation. The exposure of skin mast cells to the exuded plasma may then induce degranulation of the skin mast cells via various

receptors, releasing a massive amount of histamine, resulting in wheal formation observed in CSU.

Summary Further elucidation of the mechanism of blood coagulation and the pathway of mast cell activation by activated coagulation factors may be a target for the development of new and more effective treatments for CSU.

Introduction

Chronic spontaneous urticaria (CSU), also called chronic idiopathic urticaria (CIU), is a skin disorder characterized by almost daily recurring skin edema and flare with itch and pruritus on anywhere on the body for more than 6 weeks [1–4]. Local release of histamine from tissue-resident mast cells and/or tissue-infiltrated basophils in the urticarial lesion is considered critical for the pathogenesis of CSU. However, detailed mechanism of mast cells and basophils activation in dermis of CSU patients has been unclear [5, 6]. Several reports suggest that autoantibodies (IgGs) against IgE antibody or the high-affinity IgE receptor (FcεRI) on mast cells and basophils which induce release of histamine are detected in around 40% of patients with CSU [7, 8]. Moreover, IgE antibodies against self-molecules, such as dsDNA and IL-24, and staphylococcal enterotoxins have also been detected in patients with CSU [9–11]. The involvement of autoantigens recognized by IgE antibodies has

been suggested by the effectiveness of omalizumab (Xolair®), an anti-IgE monoclonal antibody, and association of clinical efficiency of omalizumab in CSU with a reduction of basophil FcεRI expression [12, 13]. On the other hand, infections by microorganisms, such as bacteria and/or virus, have also been suggested as an exacerbating factor in many cases of CSU [14]. Moreover, several blood molecules have been suggested as potential disease-related biomarkers to evaluate the severity and activity of CSU. The increase of D-dimer, FDP, prothrombin fragment 1+2 (PF1+2), C-reactive protein (CRP), substance P (SP), and proinflammatory cytokines including TNFα, IL-1β, IL-6, IL-17, IL-31, and IL-33 in plasma were observed in patients with CSU [15, 16, 17••, 18–20] (Fig. 1). On the other hand, a decrease of plasma level IL-35 and vitamin D was also reported [21, 22] (Fig. 1). These observations imply the involvement of these molecules in the pathogenesis of CSU.

CSU and the extrinsic coagulation cascade

The involvement of the extrinsic coagulation pathway triggered by the exposure of plasma, containing various coagulation factors, to tissue factor (TF) has been suggested in the pathogenesis of CSU [23–29]. Moreover, the effectiveness of heparin, activator of anti-thrombin which inactivate coagulation factors, such as FXa and thrombin (FIIa), and warfarin which blocks the synthesis of coagulation factors, such as FVIIa, FXa, and FIIa, in the treatment of CSU has been reported [3–32]. These studies suggest that the TF-triggered extrinsic coagulation cascade plays a causative role in the pathogenesis of CSU. Asero et al. showed that plasma levels of PF1+2 and D-dimer in patients with CSU were higher than those in healthy donors and proportional to disease severities [23–25]. PF1+2 is generated from prothrombin together with thrombin which acts on fibrinogen and generates fibrin. Fibrin is then degraded by the plasmin (fibrinolysis), resulting in the production of D-dimer and FDP. We and other

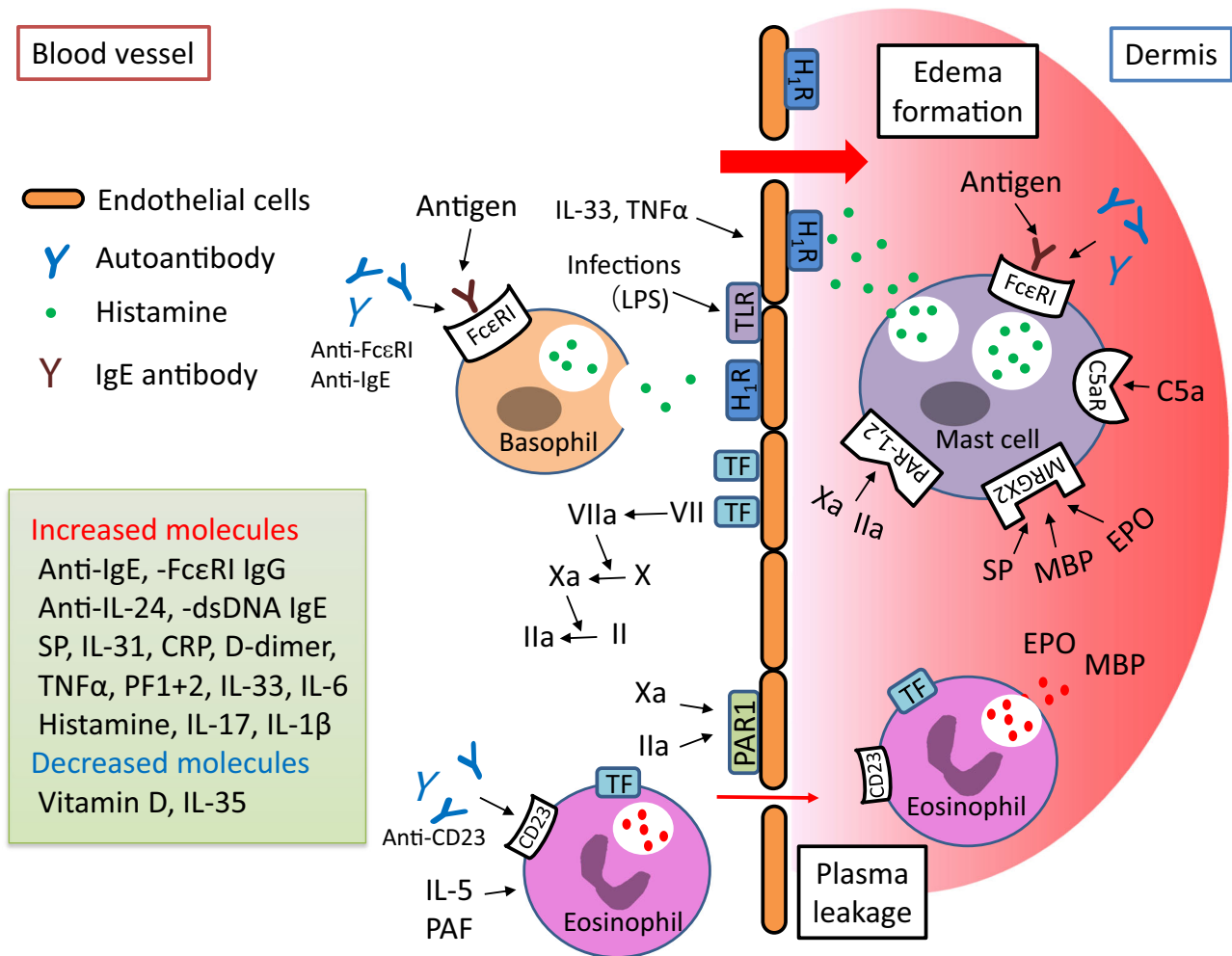


Fig. 1. Summary of the TF-triggered edema formation in CSU. TF expressed on endothelial cells and eosinophils activate the extrinsic coagulation cascade followed by leakage of plasma from blood vessel followed by the activation of mast cells resident in dermis.

groups previously reported that both levels of FDP and D-dimer in plasma of patients with CSU, but not in healthy individuals or in patients with inducible type of urticaria (IU), were increased in disease severity-dependent manner [33]. Moreover, potential of blood coagulation in the plasma of patients with CSU has been proved high [26, 27].

The extrinsic coagulation cascade triggered by TF

The expression of TF is high in highly vascularized organs such as brain, lung, and hearts [34, 35]. TF consists of an extracellular domain including FVII/VIIa binding domain, a hydrophobic transmembrane domain and a cytosolic domain for signal function [36]. When FVII in plasma binds to the extracellular region of TF, the extrinsic coagulation cascade is activated and acts together with phosphatidylserine (PS) and Ca^{2+} to produce active forms of coagulation

factors, such as FVIIa, FXa, and FIIa. Produced FIIa by the coagulation cascade then converts fibrinogen to fibrin for blood clot formation. The activated coagulation factors also activate target cells, including several inflammatory cells, via protease-activated receptors (PAR-1, PAR-2, PAR-3, PAR-4), resulting in the production and release of inflammatory mediators, angiogenesis, proliferation, and chemotaxis [36, 37].

Expression of TF on blood cells

Several kinds of blood cells, such as eosinophils and monocytes, express TF. Moosbauer et al. revealed the increase of TF on the surface of eosinophils in response to several mediators and/or cytokines, such as platelet-activating factor (PAF) and IL-5 [38]. Moreover, Asero et al. showed the enhancement of TF expression on eosinophils in the lesion of CSU [39]. Furthermore, autoantibodies against the low-affinity IgE receptor (FcεRI: CD23) which can activate eosinophils are detected in around 70% of patients with CSU [40]. Thus, TF expressed on eosinophils may be involvement of the pathogenesis of CSU. Eosinophils release major basic protein (MBP) and eosinophil peroxidase (EPO) which can induce release of histamine from mast cells via MAS-related G protein-coupled receptor member X2 (MRGX2) [41]. Therefore, eosinophils infiltrated into dermis may activate skin mast cells either by expressing TF and/or by release of MBP and EPO. Human monocytes constitutively express low levels of intracellular TF under resting conditions, but rapidly express them on the membrane in response to stimuli, such as LPS [42, 43]. Several groups have detected TF on the surface of activated human platelets [44], but the expression of TF by human platelets and neutrophils remained controversial.

Basophils do not express TF on the surface of plasma membrane. However, our previous report suggests that histamine released from basophils and LPS synergistically enhance the expression of TF on endothelial cells, followed by the activation of the extrinsic coagulation cascade [45••]. IgGs against IgE antibody and IgE receptor on basophils, which can activate basophils, were found in up to 50% of patients [7, 8, 46]. Moreover, IgE antibodies against self-molecules, such as dsDNA and IL-24 have been detected in patients with CSU [9–11]. In addition, Zheng et al. showed upregulation of NK-1 receptor, receptor for SP, on basophils and the ability of SP to induce degranulation of basophils [47]. Thus, basophils may contribute to the increase of TF expression on vascular endothelial cells. Human basophils also express PS, known as an activator of the extrinsic coagulation cascade, on their surface during degranulation (Yanase et al., unpublished observation), suggesting that basophils themselves may contribute to the activation of the extrinsic cascade in the presence of VII, TF, and Ca^{2+} .

Expression of TF on vascular endothelial cells

Several studies have suggested that histamine, $\text{TNF}\alpha$, VEGF, IL-1 β , IL-33, and TLR ligands, such as LPS, induce expression of TF on the surface of vascular endothelial cells [48–52]. However, physiological concentrations of these molecules are insufficient to induce the expression of TF for the activation of extrinsic coagulation cascade in CSU patients. We recently demonstrated that basophil-derived histamine and LPS induce synergistic expression of TF on the

plasma membrane of human umbilical vein endothelial cells (HUVECs) and human dermal microvascular endothelial cells (HMVECs) [45••]. We also demonstrated that pretreatment with LPS increased of H₁ receptor at mRNA level in HUVECs and made HUVECs more sensitive to histamine. Moreover, we elucidated that TLR-3, TLR-5, and TLR-6 agonists also induced synergistic enhancement of TF expression in HUVECs in the presence of histamine at mRNA level [45••]. Another characteristic feature of wheal in urticaria is its prompt diminishment. This phenomenon may be explained by our finding that adenosine or AMP, but not ATP or ADP, clearly terminated the expression of TF in HUVEC induced by histamine and/or LPS [45••]. ATP is released from mast cells and basophils together with histamine but shows no or only limited activity on vascular permeability by itself. Nevertheless, ATP is converted to adenosine in a step-by-step manner and suppresses the expression of TF on endothelial cells, even at 1 h or longer after the stimulation of HUVECs with LPS and histamine. Tissue factor pathway inhibitor (TFPI), which is known as a plasma Kunitz-type serine protease inhibitor and is released from endothelial cells, may also contribute to the inhibition of extrinsic blood coagulation pathway to some extent [53].

Mast cell activation in CSU

As we described above, several autoreactive IgGs such as anti-FcεRI and anti-IgE antibody which can activate mast cells have been detected in sera of patients with CSU. Moreover, several types of IgE that bind to self-molecules such as dsDNA and IL-24 have been found in patients with CSU [9–10]. Furthermore, Altrichter et al. reported the presence of IgE against staphylococcal enterotoxins in serum of patients with CSU [11]. SP is also shown to be upregulated in sera of patients with CSU, and patients with CSU appear to be sensitive to SP and VIP, ligands for MRGX2 [54]. We recently reported that neuromedin-U (NMU), which may be released from keratinocytes, also induces degranulation of human skin mast cells via MRGX2 [55•]. These findings suggest that the increase of MRGX2 ligands, such as NMU and SP, in serum may contribute to the pathogenesis of CSU as well. A recent study reported by Bossi suggested that plasma from patients with CSU causes the release of histamine from human mast cell lines, LAD2 and HMC-1 by IgE-FcεRI-independent mechanism [56]. Moreover, Cugno et al. reported the presence of molecule that is smaller than 30 kDa and induces histamine release from mast cells in patients with CSU [57].

On the other hand, PAR-2 agonists, such as trypsin, activate human mast cells [58, 59]. Moreover, the expression of PAR-2 is on human mast cells is upregulated in urticarial lesion compared with nonurticarial lesion [60]. Furthermore, Vliagoftis reported the expression of PAR-1 on bone marrow-derived mast cells (BMMCs) and its role for the adhesion of BMMCs to fibrinogen [61]. These reports imply that TF triggered the activation of coagulation factors, such as FX and II, which may then activate mast cells via PAR-1 and/or PAR-2 on the surface of mast cells, followed by the release of a large amount of histamine. Mast cells also express C5a receptor (C5aR) on the plasma membrane. Thrombin (IIa) and Xa produced by TF-triggered pathway are suggested to convert C5 in plasma to C5a which may cause activation of mast cells via C5aR [62–65]. Activated eosinophils also release various kinds of inflammatory

mediators including EPO and MBP [40], which can also provoke degranulation of mast cells through MRGX2 expressed on mast cells [41]. We recently found that adenosine also attenuate IgE-dependent activation of human basophils and skin mast cells via adenosine A2a or A2b receptor, respectively [66•]. This system may regulate the excessive activation of mast cells, resulting in central healing tendency which may be observed in wheals of CSU [67].

Mechanism of mast cell activation and wheal formation by TF-triggered coagulation cascade

The activation of skin mast cells followed by the development of urticaria via the extrinsic coagulation cascade might be explainable by following model (Fig. 1). Infections by pathogens, such as gram-negative bacteria with LPS, histamine and/or other TF-inducers, such as TNF- α and IL-33 firstly induce synergistic expression of TF on the surface of vascular endothelial cells [45••]. Mildly activated basophils in CSU patients may spontaneously release a small amount of histamine. Moreover, autoantibodies, such as IgGs against Fc ϵ RI or IgE antibody and IgE against IL-24 or dsDNA, may also contribute to spontaneous release of histamine from peripheral basophils. TF expressed on vascular endothelial cells then activates extrinsic coagulation cascade, resulting in the activation of coagulation factors, such as VII, X, and II [45••]. Xa and IIa increase vascular permeability via PAR-1 expressed on vascular endothelial cells and induce leakage of plasma containing active forms of coagulation factors. Leaked IIa and Xa to the outside of blood vessels may activate mast cells via PAR-1 and/or PAR-2 on mast cells. Meanwhile, eosinophils may be activated by autoantibodies against Fc ϵ RII, PAF, and cytokines, such as IL-5 and TNF α , and express TF. Activated eosinophils migrate outside of blood vessels and release stored inflammatory mediators, such as MBP and EPO which may induce degranulation of mast cells via MRGX2. Active forms of coagulation factors, such as Xa and IIa, may convert C5 in serum to C5a, which also induces histamine release from mast cells via C5aR. Finally, histamine released from activated mast cells in dermis by the mechanism described above should result in long and robust edema formation recognized as wheals through H₁ receptor expressed on vascular endothelial cells (Fig. 1). The reactions are then terminated by adenosine metabolized from ATP released from mast cells either by direct inhibition of mast cell activation or by withdrawal of TF expressed on vascular endothelial cells [45••].

Conclusions

We here showed a possible relationship between the expression of TF on vascular endothelial cells and/or eosinophils, and the pathogenesis of CSU via the TF-induced activation of the extrinsic coagulation cascade. Further studies on detailed mechanism of TF expression on the cells and the activation of mast cells by plasma leakage out of blood vessel in patients with CSU are necessary to clarify crucial point in the pathogenicity of CSU in a view point of better treatments. The extrinsic coagulation cascade-related factors, such as active form of coagulation factors (VIIa, Xa, or IIa), PARs (PAR-1 on endothelial cells or PAR-1, PAR-2 on mast cells), TLR antagonists and adenosine receptors

expressed on basophils, mast cells, and endothelial cells, may be effective targets for the treatment of severe and refractory CSU.

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Compliance with Ethical Standards

Conflict of Interest

Yuhki Yanase declares that he has no conflict of interest. Shunsuke Takahagi declares that he has no conflict of interest. Michihiro Hide declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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