

Molecular pathogenesis of STEC-HUS caused by endothelial heterogeneity and unprotected complement activation, leading to endotheliopathy and impaired ADAMTS13 activity: based on two-activation theory of the endothelium and vascular microthrombotic disease

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Abstract

Shiga toxin-producing *E. coli* hemolytic-uremic syndrome (STEC-HUS) is characterized by acute renal failure associated with thrombocytopenia and microangiopathic hemolytic anemia following exposure to STEC. It may present with spectrum of multi-organ dysfunction syndrome (MODS). Pathogenesis of STEC-HUS is partially understood. Proposed is two sequential molecular pathogeneses. *First path* is from Shiga toxins to endotheliopathy as a result of organotropism and endothelial heterogeneity. Shiga toxins binding to Gb3-endowed ECs, leads to endotheliopathy in the kidneys and other organs. Also, Shiga toxins downregulating CD59 provoke unprotected activation of complement, in which C5b-9 causes channel formation and promotes additional endotheliopathy. *Second path* is from endotheliopathy to microthrombogenesis. Endotheliopathy triggers activation of two independent molecular pathways (*i.e.*, inflammatory and microthrombotic), promoting inflammation and formation of “microthrombi” composed of platelet and unusually large von Willebrand factor multimers (ULVWF) complexes. Microthrombogenesis is initiated by the activated platelet and endothelial exocytosis of ULVWF. If ADAMTS13 is insufficient due to excessive production of ULVWF, uncleaved ULVWF are anchored to ECs. ULVWF anchored to Gb3-endowed and CD59-disendowed ECs *in situ* are long elongated strings and recruit the activated platelets to assemble microthrombi. Microthrombi cause disseminated intravascular microthrombosis (DIT), leading to vascular microthrombotic disease (VMTD). The result is organ phenotype syndromes of MODS such as HUS, encephalopathy, acute respiratory distress syndrome (ARDS), etc. DIT/VMTD triggers thrombotic thrombocytopenic purpura (TTP)-like syndrome. Current understanding of Gb3 and CD59 roles and experience with complement inhibition therapy support the complex molecular pathogenesis of STEC-HUS is due to heterogeneity-associated endotheliopathy, leading to impaired ADAMTS13 function.

Indexing terminology: ADAMTS13; CD59; C5b-9 [TCC: MAC]; Gb3; Complement activation; Disseminated intravascular microthrombosis; Endotheliopathy; Endothelial heterogeneity Hemolytic-uremic syndrome; Microthrombogenesis; Organotropism; Shiga toxins; TTP-like syndrome; Vascular microthrombotic disease

Abbreviations: AFH, acute fulminant hepatic failure; aHUS; atypical hemolytic-uremic syndrome; ARDS, acute respiratory distress syndrome; ARF, acute renal failure; “C”, complement; DIC, disseminated intravascular coagulation; DIT, disseminated intravascular microthrombosis; ECs, endothelial cells, HFRS, hemorrhagic fever with renal syndrome; HUS, hemolytic uremic-syndrome; MAHA, microangiopathic hemolytic anemia; MAC, membrane attack complex; MODS, multi-organ dysfunction syndrome; SIRS, systemic inflammatory response syndrome; SNP, single nucleotide polymorphism; STEC, Shiga toxin producing *E. coli*; Stx, Shiga toxin; TMA, thrombotic microangiopathy; TPE, therapeutic plasma exchange; TTP, thrombotic thrombocytopenic purpura; ULVWF, unusually large von Willebrand factor multimers; VMTD, vascular microthrombotic disease; Vtx, verotoxin

Introduction

Shiga toxin-producing *E. coli* hemolytic-uremic syndrome (STEC-HUS) is a serious kidney disease associated with intravascular

microthrombosis, resulting in organ phenotype of acute renal failure due to endothelial heterogeneity and Shiga toxin-caused organotropism. Hematologic phenotype of thrombocytopenia and microangiopathic hemolytic anemia (MAHA) is the result of impaired ADAMTS13 activity leading to disseminated intravascular microthrombosis (DIT) and thrombotic microangiopathy (TMA). The extra-renal manifestations commonly occur as the phenotypes of MODS that are associated with DIT in selected organs.

The pathogenesis of STEC-HUS is still poorly understood and its morbidity and mortality are high. Mortality is reported to be between 3% and 5%, and death due to STEC-HUS is almost always associated with severe extra-renal diseases, including serious central nervous system involvement [1].

In this brief review, the unified concept of molecular pathogenesis of STEC-HUS, leading to two sequential paths, 1) first, from STEC to endotheliopathy and 2) second, from endotheliopathy to

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microthrombogenesis, are presented as illustrated in Table 1 and Figure 1. This conceptual framework is based on “two-activation theory of the endothelium” [2].

Role of Shiga toxins

STEC-HUS is typically preceded by a prodrome of diarrhea, which is sometimes bloody, and is caused by Shiga toxin-producing *Escherichia coli* bacteria, of which *E. coli* O157:H7 is the most common serotype.

Two different Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) show the similar molecular structure. However, their role in STEC-HUS seems to be different because STEC cells could produce one or both of two major toxins, and these toxins appear to be crossing the epithelial cell barrier by different pathways [3]. Also, Stx2 is more frequently associated with severe STEC-HUS in humans for reasons as yet clearly undetermined [4-5]. Recently it was found that Shiga toxins activate complement and binds to factor H, which is a strong evidence for an active role of complement system in STEC-HUS [6].

Shiga toxins are multi-subunit protein complexes that bind to a glycosphingolipid receptor Gb3, on select eukaryotic cell types. Localization of Gb3-endowed ECs in the kidneys and other organs is predictive of the sites of action of Shiga toxins. However, the toxins are cytotoxic to some, but not to all cell types that express Gb3 [5]. Despite related primary amino acid sequences, Stx1 and Stx2 are immunologically distinct. In addition, Stx1 and Stx2 do not target exactly the same tissues and organs although both bind Gb3 and are thought to be capable of causing STEC-HUS [5]. These findings suggest the molecular pathogenesis of STEC-HUS is a complex process.

In addition, Shiga toxins were found to activate complement system [6,7]. Thus, as seen in atypical HUS (aHUS), the pathogenesis of STEC-HUS is also suspected to be complement-mediated endotheliopathy resulting in thrombotic microangiopathy [7,8]. Activation of the complement system produces terminal C5b-9 (MAC [membrane attack complex]), which insertion to unprotected ECs causes of channel (pore) formation on the membrane of ECs [9]. In order to protect themselves from the complement attack, ECs express several

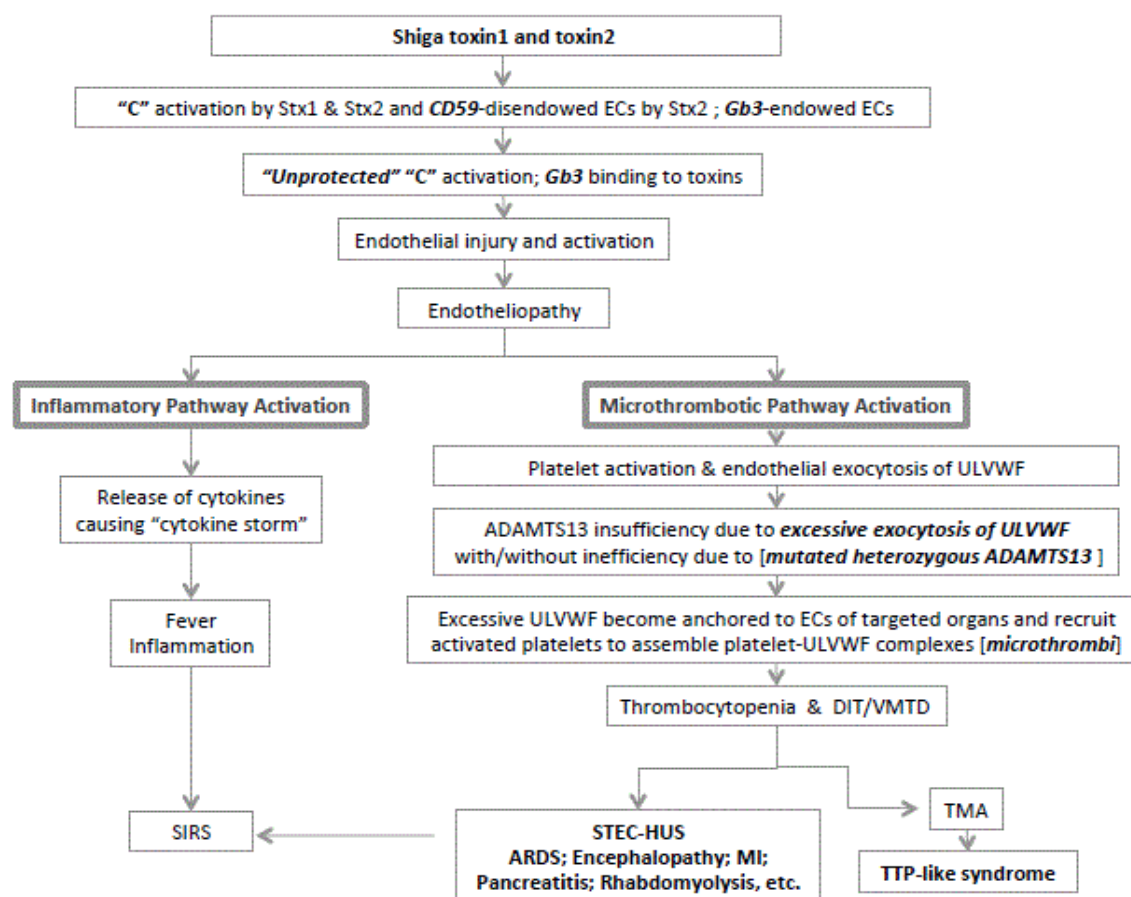


Figure 1. Molecular Pathogenesis of STEC-HUS.

Molecular pathogenesis of STEC-HUS is presented in this schematic illustration. Shiga toxins are transported to upregulated Gb3 receptors in ECs. Gb3-endowed heterogeneity of ECs is present in many different organs such as kidneys, brain, heart, pancreas, lungs, muscle, and skin, etc. The binding of Shiga toxins to Gb3 promotes endotheliopathy. Also, Shiga toxins activate complement system. Additionally, Shiga toxin2 downregulates CD59. This disendowed CD59, which is the membrane protector of ECs, contributes to “unprotected” complement activation leading to additional endotheliopathy through the channel formation in the endothelial membrane. Combined endotheliopathy activates both inflammatory and microthrombotic pathways. Excessive endothelial exocytosis of ULVWF would occur from Weibel-Palade bodies. If ADAMTS13 were insufficient due to imbalance between ADAMTS13 activity and exocytosed ULVWF with/without inefficiency due to ADAMTS13 gene mutation, uncleaved ULVWF are anchored to ECs as long elongated strings and recruit activated platelet to assemble platelet-ULVWF complexes, which are “microthrombi” that trigger endotheliopathy-associated DIT/VMTD. These multi-step processes cause TTP-like syndrome and MODS.

Table 1. Unified concept of “two-sequential pathg molecular pathogenesis the pathogenesis of STEC-HUS

Pathogenesis of STEC-HUS	First path: from STEC to endotheliopathy	Second path: from endotheliopathy to microthrombogenesis
Pathogenic source	Shiga toxins (stx1 and stx2) Shiga-like toxins (vtx1 and vtx2)	Endotheliopathy ADAMTS13 insufficiency
Gene involved	GLA CD59 “C” regulator	ADAMTS13
Modifying molecule	Gb3 (overexpressed) CD59 (underexpressed)	
Molecular mechanism	Shiga toxins binding to Gb3-endowed ECs leading to apoptosis & endotheliopathy “C” activation by Shiga toxins Shiga toxin2 causing CD59-disendowed ECs leading to “unprotected” “C” activation and channel formation & endotheliopathy	eULVWF (from endothelial exocytosis) ADAMTS13 (insufficiency) Activation of microthrombotic pathway Microthrombogenesis
Examples of target organs due to endothelial heterogeneity	Bowels; kidneys; lungs; heart; brain; pancreas; muscle; skin	ECs
Phenotype examples	Gastroenteritis/hemorrhagic enteritis; acute renal failure; encephalopathy; ARDS; acute pancreatitis; myocardial infarction; rhabdomyolysis; skin necrosis	DIT Consumptive thrombocytopenia MAHA MODS
Contemporary clinical disorder	Acute renal failure; STEC-HUS	TTP-like syndrome
Disease entity	VMTD	

regulatory molecules, including the terminal complex regulator CD59 [9] that suppresses assembly of the large MACs through inhibition of polymerization of additional C9 molecules. Since Stx2 downregulates CD59 mRNA and protein levels on tubular epithelial and glomerular endothelial cells, CD-59-disendowed ECs likely contributes to the destruction of the kidneys and other organs in STEC-HUS [10,11]. Therefore, it is suspected that STEC-HUS as well as aHUS is partly caused by “unprotected” complement activation, rather than due to the current concept of “uncontrolled” complement activation.

Endothelial heterogeneity

Microvascular ECs within different tissues and organs are endowed with distinct but as yet unrecognized structural, phenotypic, and functional attributes [12-14]. STEC-HUS is a typical example of organ phenotypic diversity related to functional molecules of ECs. For example, the distribution of CD59-disendowed ECs and Gb3-endowed ECs also regulates endothelial heterogeneity in different target organs. Diversified endothelial heterogeneity, which could be genetically determined or altered by an acquired event [5,11-12,15-19], responds to the environmental factor (*i.e.*, STEC) and contributes to diversified organ phenotypes of the disease. The extrarenal manifestations of STEC-HUS are caused by altered expression of these endowed molecules resulting in endothelial heterogeneity in various organs. As shown in Table 2, organ specific phenotypes occurring in STEC-HUS include gastroenteritis, acute renal failure (ARF), encephalopathy [20-27], acute respiratory distress syndrome (ARDS) [23,24], myocardial infarction [23,26], acute pancreatitis [25,24], rhabdomyolysis [25,27], skin necrosis [25,27], etc.

Because of organ phenotypic diversity associated with endothelial heterogeneity, in STEC-HUS, ARF could present simultaneously with encephalopathy and ARDS as a result of Shiga toxin-induced DIT. In such situation, first physician (*e.g.*, nephrologist) would call this condition “HUS” with involvement of the brain and lungs; second physician (*e.g.*, neurologist) might call it “encephalopathy” with the involvement of

the kidneys and lungs; and third physician (*e.g.* pulmonologist) call it “ARDS” with involvement of the kidneys and brain. Yet, the fourth (*e.g.*, hematologist) would call it “thrombotic thrombocytopenic purpura (TTP)-like syndrome” with MODS. Certainly, nature has created this remarkable endothelial heterogeneity and also endowed the physician with the endless perplexity and complexity of human diseases.

Molecular pathogenesis (*first path*) leading to Endotheliopathy

Shiga toxins are some of the most potent toxins among various pathogens and are known to cause an injury to ECs leading to endotheliopathy. Shiga toxins bind to the cellular receptor Gb3 that is commonly endowed to ECs [5,12,17,19,28]. These toxins bound to Gb3-endowed tubular epithelium as well as the glomerular endothelial cells, leading to cellular apoptosis and necrosis, which result in endotheliopathy. Subsequently endotheliopathy induces DIT in selected organs (*e.g.*, ARF, ARDS, and pancreatitis due to vascular microthrombosis) seen in STEC-HUS [24,26,29]. Separately, normal ECs are also endowed with CD59 [10,11,15,16], which is the membrane inhibitor of reactive cell lysis following complement activation. It might be disendowed due to gene mutation [30] or due to acquired events such as Stx2 intrusion or immune diseases [10,11,31]. This CD59-disendowed ECs could independently contribute to additional endotheliopathy through channel formation on the endothelial membrane [15,16] when complement activation produces C5b-9 (MAC).

Shiga toxins inducing the above independent molecular alterations initiate *first path* of molecular pathogenesis from STEC to endotheliopathy (Table 1). Gb3-endowed or CD59-disendowed endotheliopathy could cause functional and/or anatomical changes of various organs resulting in MODS (*e.g.*, ARF, encephalopathy, ARDS, etc.), and trigger to *second path* of molecular pathogenesis, which is from endotheliopathy to microthrombogenesis, promoting molecular process that leads to TTP-like syndrome as illustrated in Figure 1.

Table 2. Target organs in STEC-HUS associated with endothelial heterogeneity and observed clinical phenotypes.

Target organ due to endothelial heterogeneity	Organ phenotype disease	Hematologic phenotype	References of known Shiga toxin targeted organ dysfunction	Incidence of organ phenotype in STEC-HUS
Bowels	Gastroenteritis	Thrombocytopenia MAHA DIT VMTD TTP-like syndrome	Well documented	Almost 100% [25]
Kidneys	Acute renal failure		Well documented	100% [25]
Brain	Encephalopathy CNS dysfunction syndrome		[20] [21] [22] [23] [24] [25] [26] [27]	5-69% [20] [23]
Lungs	ARDS		[23] [24]	5-10% [23]
Heart	Myocardial infarction		[23] [26]	<1% [25]
Pancreas	Acute pancreatitis		[25] [24]	20% [25]
Muscle	Rhabdomyolysis		[25] [27]	<1-3% [25]
Skin	Skin necrosis		[25] [27]	<1-2% [24]
ARDS, acute respiratory distress syndrome; CNS, central nervous system; DIT, disseminated intravascular microthrombosis; MAHA, microangiopathic hemolytic anemia; STEC-HUS, Shiga toxin-induced hemolytic-uremic syndrome				

Molecular pathogenesis (second path) leading to Microthrombogenesis

According to novel thesis of “two-activation theory of the endothelium” (Figure 1) [2,32], combined Gb3 and C5b-9-induced endotheliopathy promotes activation of two independent endothelial pathways (*i.e.*, inflammatory and microthrombotic). In short, two important molecular events are: 1) release of inflammatory cytokines (*e.g.*, interleukin (IL)-1, IL-6, tumor necrosis factor- α , and others) [33,34], and 2) activation of the platelet [35] and exocytosis of ULVWF [36-38]. The former triggers inflammation through “activation of inflammatory pathway”, and the latter mediates microthrombogenesis via “activation of microthrombotic pathway”. The activation of microthrombotic pathway promotes DIT, which would lead the clinical disorder “vascular microthrombotic disease” (VMTD) [2,32,39]. VMTD is characterized by intravascular microthrombosis in various organs as seen in MODS, and hematologic features of thrombocytopenia and MAHA. DIT/VMTD might be localized in the kidneys or can become systemic by involving other target organs depending upon the distribution of heterogeneity of ECs in a particular patient.

The hematologic phenotype, which is characterized by thrombocytopenia and MAHA, is the same in different etiology-associated HUS, including 1) STEC-HUS, 2) aHUS, and 3) secondary HUS associated with infection and other critical illness/condition (*e.g.*, virus, bacteria, rickettsia, pregnancy, trauma, transplant, cancer, and drugs) [40-44]. Although some hematologists, nephrologists and clinicians would designate them as combined “TTP-HUS” syndrome, the proper diagnostic term should be “TTP-like syndrome” because it is characterized by endotheliopathy-associated DIT/VMTD [2,32,39]. The difference between TTP and TTP-like syndrome in their pathophysiological mechanisms as well as etiologies is clearly defined as shown in Tables 3 and 4 [2,32]. The tables are self-explanatory.

TTP occurs due to severe ADAMTS13 deficiency (*i.e.*, familial ADAMTS13 gene mutation or acquired antibody production against ADAMTS13), resulting in excessive accumulation of megakaryocytic ULVWF in circulation. On the other hand, TTP-like syndrome occurs at ECs *in situ* due to insufficiency of ADAMTS13 following excessive endothelial exocytosis of ULVWF in endotheliopathy with or without moderate inefficiency of ADAMTS13 (*e.g.* heterozygous gene mutation/

polymorphism) [2,32,36,39]. Megakaryocytic ULVWF triggers microthrombogenesis to cause TTP in circulation, but endothelial ULVWF, anchored to ECs, promote TTP-like syndrome.

Microthrombogenesis in endotheliopathy is the process, in which long elongated ULVWF strings are anchored to ECs after release from Weibel-Palade bodies and recruit activated platelets, promoting the formation of platelet-ULVWF complexes [45-47]. It occurs when ADAMTS13 is insufficient to cleave the excessively exocytosed ULVWF. Platelet-ULVWF complexes are “microthrombi”, which lead to DIT and VMTD [2,32] in CD59-disendowed and Gb3-endowed ECs of certain vital organs.

Organotropism

Organotropism of pathogens occurs due to their metabolic versatility and ability to produce diverse bioactive compounds such as hydrolytic enzymes, antibiotics, anti-tumorals and also toxins [48]. Toxins can be produced by prokaryotes such as bacteria [1], but also by eukaryotes such as fungi [48].

In *first path* of molecular pathogenesis of STEC-HUS, renal organotropism of Shiga toxins is expressed by their affinity to ECs in the kidneys and other organs that are associated with unique endothelial heterogeneity characterized by distribution of endowed/disendowed molecules. Shiga toxins appear to trigger combined endotheliopathy in association with Gb3 upregulation and CD59 downregulation in ECs.

The virulence of Shiga toxin might be expressed by organotropism (*e.g.*, brain vs. kidneys) and combined role of molecular effects of Gb3 binding and CD56-unprotected complement activation (*e.g.*, STEC-HUS vs. a HUS). Also, *second path* of molecular pathogenesis associated with the expressivity of ADAMTS13 gene mutation on microthrombogenesis could play a role to the degree of virulence of Shiga toxins in producing hematologic phenotype of TTP-like syndromes.

Endothelial heterogeneity and pathogen/toxin-induced organotropism are two sides of the same coin in the molecular pathogenesis. It should be emphasized that HUS is not a specific syndrome by itself, but is only the manifestation (*i.e.*, ARF) from the localization associated with endothelial heterogeneity.

Thus, the proper term for the variety of *TTP-syndromes* (*i.e.*, thrombocytopenia, MAHA and various organ phenotypes) can be defined as follows with designation of *prominent organ failure*: for examples,

Table 3. Genesis of VMTD and characteristics of TTP and TTP-like syndrome.

	Hereditary TTP: ADAMTS13 gene mutation-associated VMTD Acquired TTP: ADAMTS13 antibody-associated VMTD	TTP-like syndrome: Endotheliopathy-associated VMTD
Primary event (causes)	Hereditary ADAMTS13 gene mutation Acquired ADAMTS13 antibody formation	Pathogen (e.g., viruses; bacteria; fungi; rickettsia; parasites) Polytrauma (e.g., chest/lung; bones; skull/brain injury) Pregnancy (e.g., preeclampsia; abruptio placenta; amniotic fluid embolism) Cancer (e.g., stomach; breast; lung) Transplant (e.g., liver; kidney; bone marrow) Drug and chemical (e.g., cyclosporine; mytomyacin C; Shiga toxins)
Secondary event	Excessive circulating mULVWF	Complement activation (C5b-9 production) → endothelial injury → endotheliopathy
Tertiary event	Microthrombogenesis leading to platelet-mULVWF complexes in circulation	Cytokine release → Inflammation → SIRS Endothelial exocytosis of eULVWF & anchored to ECs as long elongated strings → DIT
Final event	Microthrombi lodged in arteriolar capillary lumens of organs ↓ VMTD ↓ TMA (microthrombotic microangiopathy) ↓ TTP	Microthrombogenesis leading to platelet-eULVWF complexes anchored to ECs ↓ VMTD ↓ TMA (microthrombotic angiopathy) ↓ TTP-like syndrome
Hematologic features Platelet Red blood cell	Consumptive thrombocytopenia MAHA	Consumptive thrombocytopenia MAHA
Clinical syndromes Inflammation Cytokine storm SIRS Encephalopathy ARDS AFH ARF/HUS “DIC” (see Ref. 2 & 32)	Uncommon Absent Absent Very common Probably absent Probably absent Very common Doesn't occur	Very common Often present in sepsis and MODS Often present in sepsis and MODS Common, especially in HUS Common Common, sometimes with hepatic coagulopathy Common Identical to TTP-like syndrome
Laboratory features ADAMTS13 activity ADAMTS13 antibody LDH Haptoglobin Schistocytosis	Markedly decreased (<5% of normal) Positive in acquired TTP Increased Markedly decreased ++ to ++++	Mild to moderately decreased (20-70% of normal) or normal Negative Increased Markedly decreased None to +++
Therapeutic response to TPE Platelet transfusion rADAMTS13	Very good response Contraindicated Unknown at this time; expected to be effective in hereditary TTP	Excellent and fast response if treated in early stage Contraindicated Unknown at this time; expected to be very effective in non-infectious conditions

AFH, acute fulminant hepatitis; ARF/HUS; acute renal failure/ hemolytic-uremic syndrome; ARDS, acute respiratory distress syndrome; “DIC”, disseminated intravascular coagulation of McKay; ECs, endothelial cells; eULVWF/mULVWF, endothelial unusually large von Willebrand factor multimers/megakaryocytic ULVWF; LDH, lactate dehydrogenase; MAHA, microangiopathic hemolytic anemia; rADAMTS13, recombinant ADAMTS13; SIRS, systemic inflammatory response syndrome; TMA, thrombotic microangiopathy; TPE, therapeutic plasma exchange; TTP, thrombotic thrombocytopenic purpura; VMTD, vascular microthrombotic disease

- TTP-like syndrome with ARF (including HUS, hepato-renal syndrome, hemorrhagic fever with renal syndrome (HFRS) due to hanta virus)
- TTP-like syndrome with encephalopathy (including HUS, hepatic encephalopathy)
- TTP-like syndrome with acute fulminant hepatic failure (AFH) (including HELLPs)
- TTP-like syndrome with ARDS (including cardio-pulmonary syndrome)
- TTP-like syndrome with acute adrenal insufficiency (including Warter house-Friderichsen syndrome, septic shock)
- TTP-like syndrome with stroke syndrome (including transient ischemic attack)
- TTP-like syndrome with pancreatitis
- TTP-like syndrome with rhabdomyolysis
- TTP-like syndrome with myocardial infarction
- TTP-like syndrome with sepsis syndrome (including “TTP-mimicking syndrome”)
- TTP-like syndrome with peripheral digit ischemic syndrome
- TTP-like syndrome with “DIC” (disseminated intravascular coagulation) [2,32]

Table 4. Characteristics of two different ULVWF.

	mULVWF	eULVWF
Synthesized in	Megakaryocytes	Endothelial cells
Stored in	α granules of platelets	Weibel-Palade bodies of ECs
Primary distribution at release	In circulation	On the membrane of ECs
Availability	In blood	At activated ECs after endothelial exocytosis
Exposure to ADAMTS13	As platelet-adherent form	As ECs-adherent form
Interaction with platelets causing	Platelet aggregation and adhesion	Platelet recruitment and aggregation
Localization of platelets-ULVWF complexes	Arteriolar capillary lumens lodged as microthrombi	Intravascular ECs-anchored as platelet-decorated long elongated ULVWF strings
Example of its attachment to	Auto-ADAMTS13 antibody	ECs in sepsis-induced endotheliopathy
TMA manifestation	Microthrombotic microangiopathy	Microthrombotic angiopathy
Hematopathologic manifestation	Thrombocytopenia MAHA Encephalopathy; ARF	Thrombocytopenia MAHA MODS (ARF; HUS; encephalopathy; ARDS; MI)
Associated inflammation	None to minimal (?)	None to severe
Associated clinical syndrome	TTP	TTP-like syndrome

- TTP-like syndrome with MODS (multi-organ dysfunction syndrome) and so on.

VMTD in TTP and TTP-like syndrome

Succinctly speaking, TTP is either gene mutation-associated (hereditary) or antibody-associated (acquired) DIT/VMTD. TTP is well defined. In contrast, TTP-like syndrome is endotheliopathy-associated DIT/VMTD [2,32], which clinical manifestation is associated with endothelial heterogeneity. Thus, TTP-like syndrome is well defined too. Better understanding of distinct nature of molecular pathogenesis of TTP-like syndrome clearly explains how TTP-like syndrome is different from TTP. The same hematologic phenotype is probably due to the similar nature of “microthrombi” composed of platelet-ULVWF complexes (Table 3). It is appropriate to call both disorders as VMTD.

Hematologic phenotype of thrombocytopenia and MAHA is almost identical in both TTP and TTP-like syndrome. Because TTP occurs in circulation and TTP-like syndrome occurs at the endothelial membrane in association with endothelial heterogeneity, the organ phenotype is dissimilar. TTP typically tends to involve in the kidneys and brain, which is because circulating microthrombi become easily lodged in those organs, but microthrombogenesis in TTP-like syndrome develops at ECs.

In STEC-HUS, if the target organ is localized and confined only to the kidneys, the effect of activation of inflammatory pathway is muted, which would cause minimal inflammation if ever present. However, if endothelial heterogeneity is significantly expressed not only in the kidneys, but also other organs [11,21,49], moderate to severe inflammation, including systemic inflammatory response syndrome (SIRS), could develop [49-51]. According to “two-activation theory of the endothelium”, inflammation is typically absent in TTP because activation of inflammatory pathway is not incited in the absence of endothelial response.

Insight on the molecular pathogenesis from therapeutic experiences

First, because of the same hematologic phenotypic feature of HUS to TTP, therapeutic plasma exchange (TPE) has been employed and evaluated for both STEC-HUS and aHUS. There is insufficient evidence of benefit with TPE in the treatment of Shiga toxin-mediated disease [52]. For aHUS, TPE had been the mainstay of treatment prior to the use of eculizumab [53] and has been thought it might be helpful especially for the patients with no identified complement mutation or in whom TTP is suspected [54]. Although TPE has reduced the high mortality rate and prevented relapses, it is far from optimal.

Second, because complement inhibitor eculizumab has been used successfully, it is presumed “uncontrolled” complement activation is associated with complement mutation. Eculizumab is now recognized as the treatment of choice for aHUS [55,56]. However, the benefit of eculizumab and/or TPE for STEC-HUS should be established with well-designed clinical trials. Some cases reports have suggested significant benefits with eculizumab [57-59]. An analysis of registry data presented at the XLIX European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Congress suggested that the combination of eculizumab with TPE for treating the accompanying STEC-HUS is no better than TPE alone [60]. In a review of the therapy for HUS, complement inhibition was effective in most patients with aHUS but was beneficial only in some patients with STEC-HUS or secondary HUS [61].

Although complement clearly contributes to the molecular pathogenesis in patients with STEC-HUS [62], only limited patients seem to benefit from early administration of eculizumab. Most of STEC-HUS patients do not respond to this drug [60]. These conflicting findings can be explained by the “combined two-leg two-path molecular pathogenesises” of STEC-HUS. In *first path*, combined (two-leg) Gb3-endowed ECs, which is complement independent, and CD59-disendowed ECs, which is complement dependent, Gb3-induced endotheliopathy could be the

culprit for poor response. Gb3-induced endothelial damage would not benefit from complement inhibition alone. This is the critical difference between STEC-HUS and aHUS. Eculizumab would work only in the earlier stage of complement dependent aHUS. Once the hematologic phenotype of TTP-like syndrome is diagnosed, aHUS is in the *second path* and microthrombogenesis already must have started and is in advanced stage. In this case, TPE could be more beneficial treatment for DIT/VMTD.

Potential design of molecular therapy for STEC-HUS

According to proposed molecular pathogenesis of STEC-HUS, *first path* is caused by organotropism of Shiga toxins attracting to CD59 and Gb3-associated endothelial heterogeneity, and *second path* is caused by microthrombogenesis, leading to DIT, renal necrosis and end stage renal diseases in sequence. Theoretically, *first path* should be treated with Gb3 inhibition (e.g., anti-Gb3 antibody) and complement inhibition using anti-C5 (eculizumab) or CD59 replacement (e.g., recombinant CD59) if the disease is in very early stage. However, the better target is *second path*, which molecular pathogenesis should be treated with anti-microthrombotic therapy (e.g., TPE), but perhaps better yet recombinant ADAMTS13.

For the practicality, it is impossible to begin timely treatment for STEC-HUS to prevent both *first path* and *second path*. If thrombocytopenia and MAHA were recognized with advanced DIT and organ necrosis, the benefit targeting *first path* is long passed and *second path* would be in advancing stage. Thus, the chance of recovery may be not very high. Since full-blown DIT is the crux of problem leading to irreversible end stage renal disease and other vital organ involvement, antimicrothrombotic therapy using recombinant ADAMTS13 (currently only approved for clinical trial of hereditary TTP) might be more effective molecular therapy.

Theoretically, the best approach should be the initiation of anti-microthrombotic therapy at the earliest possible time. This can be accomplished by close monitoring of platelet count and intravascular hemolysis for the patient during the outbreak of STEC, especially when presented with gastroenteritis. Unexplained thrombocytopenia and intravascular hemolysis with gastroenteritis should confirm the diagnosis of STEC-HUS and be the indication for immediate treatment with TPE at this time. Since STEC infection is self-limited disease, the better treatment may be with prophylactic recombinant ADAMTS13 administration if it is approved.

Complement inhibition therapy and CD59 replacement trial should be contraindicated if the patient is suspected to have any kind of coexisting infection or is immunosuppressed. The discussion of this issue is not the scope of this article.

Conclusion

One environmental factor and several mutated genotypes participate in the molecular pathogenesis of STEC-HUS. The severity of STEC-HUS depends upon the endotheliopathy related to: 1) Shiga toxin organotropism, 2) endothelial heterogeneity and 3) functionality of ADAMTS13 gene. Shiga toxins are the initiators via “unprotected complement activation” and Gb3 binding, endothelial heterogeneity is the fabricator of organ phenotypes, and ULVWF are the molecular mediator of microthrombogenesis leading to TTP-like syndrome. These several molecular ingredients make up the complex nature of STEC-HUS.

STEC-HUS is the prototype of TTP-like syndrome revealing how interaction takes place between an environmental factor and mutated

gene(s). Endotheliopathy-associated DIT/VMTD (*i.e.*, TTP syndromes) should be a good place to study the nature of endothelial heterogeneity in many human diseases.

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