Possible Mechanism of Hemolytic Uremic Syndrome (HUS) Development after Infection of *E. Coli* O157:H7

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Hemolytic uremic syndrome (HUS) is characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure. As evidenced in the 1996 epidemic of Escherichia coli O157:H7 in Japan, involving more than 9,000 people, HUS develops several days after an initial diarrheal illness caused by verotoxin (VT)-producing E. coli . Patients with a mild form of the disease then develop symptoms relating to anemia, thrombocytopenia and renal failure. Severely affected patients proceed to develop multi-organ disease including damage of the myocardium, pancreas, gut, liver and kidney. VT-mediated damage to the vascular endothelium is believed to cause a procoagulant state resulting in occlusion of microvessels, including renal glomerular cells. However, clear evidences have not been revealed. The histology of HUS shows a glomerular thrombotic microangiopathy in which the thrombi contain fibrin as well as platelet elements, unlike the platelet predominant thrombi of thrombotic thrombocytopenic purpura (TTP). This local fibrin deposition suggests activation of coagulation and/or a decrease in fibrinolytic potential.

E. coli O157:H7 isolated from HUS patients produces both VT-1 and VT-2. VT-1 is identical with Shiga toxin produced from Shigella dysenteriae, whereas VT-2 differs from Shiga toxin in immunological properties. VT-1 is a 69 kDa complex of proteins comprised of five b-subunits (7 kDa each) and one α -subunit (30 kDa). The VT-1 holotoxin binds via the b-subunit pentamer with high affinity to the terminal digalactose of glycosphingolipid globotriaosylceramide (Gb3) which is believed to be a cellular receptor for VT-1. Vascular endothelial cells and monocytes expressing Gb3 are sensitive to VT-1 and the toxin are internalized after binding with the receptor.

Vascular endothelial cells participate in the regulation of hemostasis through controlling the expression of anti- and pro-coagulant factors. Thrombomodulin is a high affinity thrombin receptor expressed on the surface of endothelial cells

and one of major biologic significance for antithrombotic properties on endothelial cells. Heterogenous soluble forms of thrombomodulin antigen have been detected in circulating blood, which are composed of 6-7 fragments of native thrombomodulin. The release of thrombomodulin antigen from the endothelial cells into cultured medium was negligible small, even if the cells were stimulated by several agonists, whereas a marked increase in the antigen levels was observed when the cells were injured by treatment with activated neutrophils or active oxygen. Therefore, it has been assumed that the soluble thrombomodulin antigen are produced by the proteolytic fragmentation of native thrombomodulin expressed on the surface of endothelial cells through injury to the cells. The present work demonstrated that VT-1 induced release of soluble TM fragments from cultured human umbilical vein endothelial cells (HUVECs) to cultured medium in vitro and an increased level of TM fragments was observed in the plasma/serum of patients developed with HUS after infection of E. Coli O157, suggesting the injury of endothelial cells by VT-1.

Tissue factor (TF) is of major biologic significance for procoagulant properties in endothelial cells and monocytes and functions as a membrane glycoprotein receptor that specifically binds coagulation factors VII or VIIa. TF functions as a cofactor for VIIa-dependent activation of coagulation factors IX and X, thereby initiating the coagulation cascade. Under normal circumstances, endothelial cells and monocytes either do not express TF activity or express very low activity. Thus, the normal vascular endothelial cells and monocytes are relatively inert with respect to initiating coagulation. The induction of TF expression represents an elevation of procoagulant properties in circumference of the cells including plasma. The present work demonstrated that VT-1 stimulated TF expression with increase in TF mRNA levels in cultured HUVECs and monocytes. The 5'-flanking region of TF gene contains the NF-kB, AP-1, Egr-1 and Sp-1 binding sites. The increased levels of TF mRNA accompanied with the increased bindings of p65/p50 NF-kB to TF-kB oligonucleotides and AP-1 containing c-Jun and JunD to the proximal AP-1 motif of TF promoter. Therefore, the present work suggests that VT-1 injures vascular endothelial cells and induces TF expression through increased bindings of the activated NF-kB and AP-1 to the TF-kB and proximal AP-1 binding sites of the TF promoter in the injured endothelial cells and monocytes accumulated in the injured regions. These actions of VT-1 could participate to form the fibrin-rich thrombi observed in patients infected with E. coli O157:H7 and develop HUS and multi-organ disease.