Human mannose-binding lectin inhibitor prevents Shiga toxin–induced renal injury

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Hemolytic uremic syndrome caused by Shiga toxin–producing *Escherichia coli* (STEC HUS) is a worldwide endemic problem, and its pathophysiology is not fully elucidated. Here we tested whether the mannose-binding lectin (MBL2), an initiating factor of lectin complement pathway activation, plays a crucial role in STEC HUS. Using novel human MBL2-expressing mice (MBL2 KI) that lack murine Mbls (*$MBL2^{+/+}Mbl1^{-/-}Mbl2^{-/-}$*), a novel STEC HUS model consisted of an intraperitoneal injection with Shiga toxin-2 (Stx-2) with or without anti-MBL2 antibody (3F8, intraperitoneal). Stx-2 induced weight loss, anemia, and thrombocytopenia and increased serum creatinine, free serum hemoglobin, and cystatin C levels, but a significantly decreased glomerular filtration rate compared with control/sham mice. Immunohistochemical staining revealed renal C3d deposition and fibrin deposition in glomeruli in Stx-2–injected mice. Treatment with 3F8 completely inhibited serum MBL2 levels and significantly attenuated Stx-2 induced–renal injury, free serum hemoglobin levels, renal C3d, and fibrin deposition and preserved the glomerular filtration rate. Thus, MBL2 inhibition significantly protected against complement activation and renal injury induced by Stx-2. This novel mouse model can be used to study the role of complement, particularly lectin pathway-mediated complement activation, in Stx-2–induced renal injury.

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Hemolytic uremic syndrome (HUS) is a disease characterized by renal failure, thrombocytopenia, and hemolytic anemia.[1] HUS is divided into the following 2 major classes by its etiologies: Shiga toxin-2 (Stx-2)–producing *Escherichia coli* HUS (STEC HUS) and atypical HUS (aHUS). Stx, an AB5 toxin produced by bacteria, has been identified as the causative factor in STEC HUS.[2] A major source of Stx is ingestion of food contaminated with Stx-producing *Escherichia coli* or *Shigella dysenteriae*.[3] Currently there is no proven therapeutic option for STEC HUS other than supportive care.[4] Supportive therapy with dialysis in the acute phase of STEC HUS can be life-saving; however, chronic renal failure and hypertension develop in many patients.[5,6] Establishment of definitive therapy to treat STEC HUS is needed to decrease mortality and morbidity.

Stx is a member of the ribosome-inactivating proteins and is classified into the following 2 main forms: Stx-1 and Stx-2.[7] Stx-2 is associated with severe human disease.[8] The B subunits of Stx bind to a glycosphingolipid receptor, globotriaosyl ceramide (Gb3), which is expressed on the plasma membrane, and the receptor-bound Stx is then internalized to the cytosol. Gb3 is highly expressed in human renal endothelial cells, and cellular injury may occur after Stx exposure.[9] Stx is then transported to the ribosome where the A subunit cleaves an adenosine at position 4324 from the 5′ terminal of the 28 S ribosome and leads to protein synthesis inhibition and cell injury.[10] In a small number of instances, protein synthesis may be enhanced (e.g., tissue factor).[11,12] Although these intracellular mechanisms have been elucidated, the mechanisms by which ribosomal inactivation induces the peculiar characteristics of STEC HUS are unknown.[13]

Involvement of the complement system has been suggested as a possible mechanism in the development of STEC HUS.[14] Low C3 plasma levels in STEC HUS patients have been reported.[15,16] Other changes in the complement system include increased levels of breakdown products of the 2 components of the alternative pathway C3 convertase, C3 (C3b, C3c, C3d), factor B (Ba and Bb), as well as sC5b-9.[17–19] These findings imply that inhibiting the complement system may attenuate the cell injury and lead to resolution of renal insufficiency. In children with severe neurological STEC HUS, anti-C5 antibody eculizumab was shown to be clinically

effective.[20] In contrast, other studies have questioned the effectiveness of eculizumab in the treatment of STEC HUS.[21,22] However, the role of the lectin complement pathway in STEC HUS is unknown. We hypothesized that mannose-binding lectin 2 (MBL2) of the lectin pathway plays a role in the pathogenesis of STEC HUS.

MBL2 is an initiating factor of the lectin complement pathway and recognizes pathogens and altered-self cells, resulting in induction of an inflammatory response and thrombogenesis. On MBL2 binding to its target, serine proteases called MBL/ficolin-associated serine proteases (MASP-1, MASP-2, MASP-3) activate C4 and C2 leading to formation of the C3 and C5 convertases and ultimately to formation of the membrane attack complex (e.g., C5b-9). MASPs are also involved in cleavage of prothrombin to thrombin, cleavage of fibrinogen to fibrin, and activation of factor XIII.[23,24] MBL2 and MASPs are involved in the activation of multiple biological pathways involved in thrombotic diseases.[25] We have shown that MBL2 is associated with endothelial injury during sterile injury such as ischemia/reperfusion injury and thrombogenesis.[26]

To investigate the role of MBL2 further in clinically relevant settings, we recently described a mouse model in which human MBL2 is knocked-in (KI) with the absence of murine MBLs ($MBL2^{+/+}Mbl1^{-/-}Mbl2^{-/-}$; MBL2 KI mouse) and also used a functionally inhibiting monoclonal antibody (mAb) against MBL2, clone 3F8[27–29] to inhibit MBL2 function. To mimic STEC HUS, we modeled Stx-induced renal injury using the MBL2 KI mouse and purified Stx-2. In the current study, we examined the role of MBL2 in the pathogenesis of STEC HUS and explored the effectiveness of 3F8 in this mouse model.

RESULTS

Determination of Stx-2 and 3F8 doses

We determined the toxicity of Stx-2 in MBL2 KI mice. Dose-response experiments determined that the 50% lethal dose was 625 pg/g. Administration of Stx-2 (625 pg/g) resulted in rapidly progressive weight loss; mice were moribund 3 days later and displayed convulsions and ataxia, indicating impairment of the nervous system. To reproduce more clinically relevant STEC HUS in MBL2 KI mice without death, the Stx-2 dose was reduced to 125 pg/g. We used weight loss as an indicator of toxicity instead of death in these pilot studies because weight loss in rodent STEC HUS models correlates with illness.[30–32] With a dose of Stx-2 (125 pg/g), various degrees of weight loss developed in MBL2 KI mice, but none exhibited neurological deficits by day 4. This sub-lethal dose of Stx-2 (125 pg/g) was used in all experimental studies. For sham/controls, mice were injected with either phosphate-buffered saline (PBS) or toxoid (e.g., a mutated form of Stx-2 that is produced and purified in the same manner as Stx-2).[33,34]

Mice were weighed every 24 hours from day −1 to day 4 (Figure 1). The MBL2 KI mice receiving Stx-2 and an isotype control monoclonal antibody (clone 1C10) began to lose body weight by day 2. On day 4, these mice lost an average of 11% of their baseline body weight. To control for any contaminating lipopolysaccharide or blood volume changes, we injected mice with toxoid or PBS.[33,34] Similar to PBS, toxoid-injected mice had no weight loss over the 4-day observation period. MBL2 KI mice treated with 3F8 and receiving Stx-2 had significantly less weight loss at day 4 compared with the 1C10 + Stx-2 group.

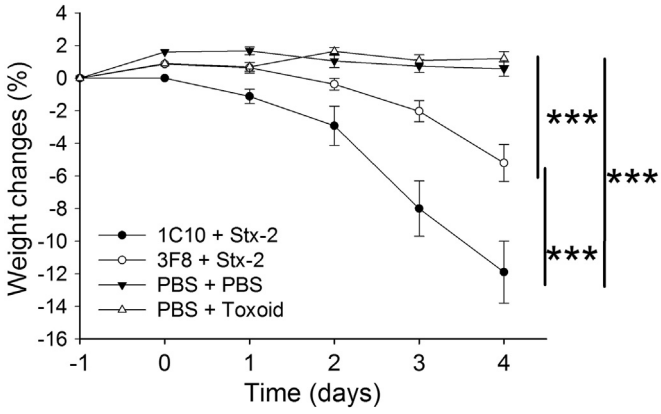

Figure 1 | Time course of weight changes. Baseline weight measurements were obtained at day −1 followed by injections on days 0.5 and 0 for the following groups: Stx-2 + 1C10 ($n = 5$), Stx-2 + 3F8 ($n = 30$), PBS + PBS ($n = 7$), and PBS + toxoid ($n = 5$). ***$P < 0.001$. PBS, phosphate-buffered saline; Stx-2, Shiga toxin-2.

Functional serum MBL2 levels were evaluated in mice receiving PBS or toxoid and mice receiving Stx-2 and treated with either PBS, 1C10, or 3F8 (Figure 2). All groups had similar levels of circulating MBL2 (e.g., ∼3 μg/ml of sera), which was significantly inhibited in mice receiving 3F8.

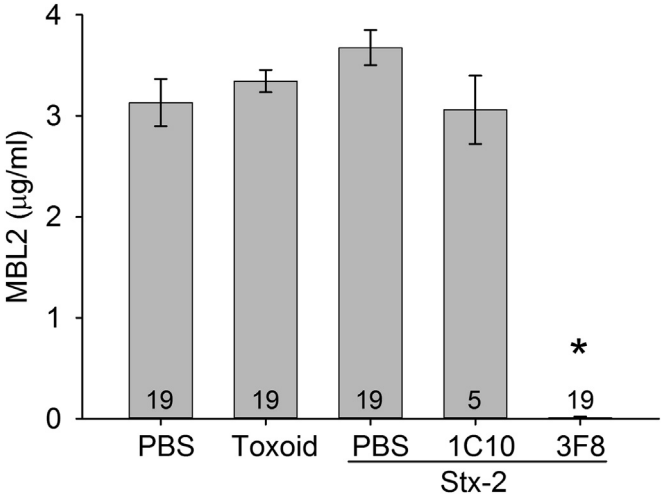

Figure 2 | Serum MBL2 concentrations in MBL2 KI mice on day 4. MBL2 concentrations remained at baseline levels after 4 days in all groups, except those treated with the anti-MBL2 mAb 3F8, which completely inhibited circulating MBL2 levels. Data are shown as the mean ± SEM. Numbers within the bars represent the number of animals studied. *$P < 0.001$ comparing 3F8 + Stx-2 group with all other groups. KI, knockin; mAb, monoclonal antibody; MBL2, mannose-binding lectin; PBS, phosphate-buffered saline; Stx-2, Shiga toxin-2.

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