NF-κB and neutrophils in post-diarrheal HUS

Post-diarrheal hemolytic uremic syndrome (D+ HUS), the most common cause of acute renal failure in infants and young children, is a substantial cause of acute mortality and chronic morbidity in this age group [1], and the outcome for adults is even worse [2]. There are no specific treatments of proven value and care continues to be merely supportive. Greater insight into the pathogenesis of this often-devastating disease is sorely needed as a prelude to developing effective therapies.

The development of D+ HUS involves a complex interaction between circulating lipopolysaccharide (LPS) and Shiga toxin (Stx) and locally produced factors that promote and/or regulate inflammation and thrombosis. Integral to the elaboration of these disease mediators is nuclear factor-kappa B (NF-κB), a family of transcription factors that include NF-κB1 (p50) and p65 (RelA), which were studied in the report of Zoja and associates in this issue of Kidney International [3]. NF-κB normally exists in cytoplasm bound to inhibitory proteins (IκB), but can be rapidly liberated in response to a variety of stimuli via a complex signaling pathway that involves phosphorylation of IκB by IκB kinases (IKK) followed by proteasome degradation [4]. The biologically active NF-κB then translocates into the nucleus and initiates expression of genes that contain the NF-κB nucleotide recognition sequence. Among the stimuli that can initiate kinase-mediated phosphorylation, those that appear to be most relevant to D+ HUS are lipopolysaccharides (LPS) [5], cytokines (for example, tumor necrosis factor-α (TNF-α)) [4], oxidative stress [6], shear stress [7], and Stx [3, 8].

Lipopolysaccharide elaborated in the gut by enterohemorrhagic E. coli is absorbed into the systemic circulation and very likely contributes to the pathogenesis of the renal injury. Antibodies to LPS can be demonstrated in flow chamber, and that leukocyte adhesion is amplified in up to 90% of patients who have had D+ HUS [12] or LPS [13]. Sakiri, Ramegowda and Tesh have shown that Stx1-induced TNF-α activation in a human monocytic cell line was preceded by nuclear translocation of NF-κB and activator protein-1 (AP-1), and loss of cytoplasmic IκB-α [8]. Evidence of oxidative stress in children with D+ HUS comes in the form of elevated blood levels of markers of lipid peroxidation [14]. NF-κB is activated by oxidative stress, and its activation can be inhibited by antioxidants [15]. However, there is little evidence that oxidative stress plays a central role in NF-κB activation, and its role in activation of NF-κB appears to be stimulus and cell specific [6].

Shear stress occurs in the renal microvasculature, particularly at vascular branches or bifurcations [7]. It would be expected to be higher in microvessels experiencing increased turbulence as might occur subsequent to thrombotic microangiopathy (TMA). Shear stress is thought to activate NF-κB and rapidly up-regulate genes encoding endothelial cell adhesion molecules [such as, vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM)], hemostatic factors [such as, tissue plasminogen activation (tPA), plasminogen activator inhibitor-1 (PAI-1)], chemoattractant factor [such as monocyte chemoattractant factor-1 (MCP-1)], and vasoactive substances [such as, endothelin, endothelial cell-nitric oxide synthase (ec-NOS)] [7, 16]. Morigi and associates have shown that Stx1 induces leukocyte adhesion to cultured endothelial cells subjected to shear stress in a parallel flow chamber, and that leukocyte adhesion is amplified by TNF-α and mediated by up-regulation of adhesive proteins (E-selectin, ICAM-1, VCAM) [17]. The mechanisms by which shear stress activates NF-κB are incompletely understood, but probably relate to conformational changes in proteins within the cytoskeleton [16].

Shiga toxin is a recently recognized stimulus to NF-κB activation. Sakiri and associates, using cultured human monocytes, have shown that Stx1 induces phosphorylation and degradation of IκB and subsequent nuclear translocation of transcription factors NF-κB and AP-1 [8]. In this issue of Kidney International, Zoja and associates have, in an elegant series of experiments, extended the NF-κB stimulating properties of Stx to cultured human endothelial cells [3]. Stx2 markedly enhanced both adher-
ence to and transmigration of leukocytes across cultured human umbilical vascular endothelial cells (HUVECs) and human glomerular endothelial cells (HGEC) exposed to hemodynamic stress of a magnitude one might expect to find in the renal microvasculature. Antibody blocking of the chemokines (IL-8 and MCP-1) that drive recruitment and activation of neutrophils and monocytes, respectively, inhibited adhesion and transcellular transmigration. Moreover, overexpression of IκB-α (the cytoplasmic protein that normally inhibits NF-κB), produced by transfecting endothelial cells with adenovirus encoding for IκB-α, essentially blocked NF-κB mediated up-regulation of IL-8 and MCP-1, and markedly inhibited adhesion and transmigration of neutrophils and monocytes, respectively.

There is considerable circumstantial evidence that leukocytes participate in the pathogenesis of D+ HUS. The magnitude of leukocytosis during the prodromal colitis predicts the likelihood of progression to HUS [18]. Moreover, leukocytosis is a feature of D+ HUS, and the degree of leukocytosis at the onset of HUS correlates with severity and outcome [19]. Serum and urinary concentration of MCP-1 and IL-8 are elevated in HUS patients, and values are highest in those who develop anuric renal failure [20], and the highest IL-8 values are found in those who die during the acute phase of D+ HUS [21]. At time of hospitalization for D+ HUS, neutrophils show reduced expression of CD16 and CD11b, are degranulated, and have impaired antibody-dependent cellular cytotoxicity that normalizes at recovery [22]. This suggests that neutrophil activation is early and transient. There also are increased numbers of neutrophils in glomeruli of fatal cases of HUS compared to normal controls [23]. These clinical observations are corroborated by experimental data; Stx induces release of superoxide from neutrophils [24]. Moreover, in a primate model of HUS [13], the coadministration of Stx1 and LPS markedly increases urinary and plasma IL-8 concentrations and renal neutrophil infiltration; in a murine model, Stx2 causes neutrophilia and PMN CD11b expression (both of which are amplified by pretreatment with LPS), as well as enhanced neutrophil adhesion, and greater neutrophil cytotoxicity [25].

There is considerable interest in developing drugs that block NF-κB–mediated disease, but one must be circumspect in inhibiting a pathway that is critical for so many immunological functions that are required for the normal host response to infections [10]. Moreover, NF-κB not only amplifies the inflammatory response to Stx, but also may be important in its resolution, probably by mediating apoptosis of neutrophils [26]. In the meantime, observations like those of Zoja and associates bring us closer to an understanding of this complex pathogenic cascade. Only through an understanding of the early molecular and cellular events in D+ HUS will we be able to identify novel molecular targets for therapeutic intervention.