


**Targeting Complement C5 in Atypical Hemolytic Uremic Syndrome**

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Hemolytic uremic syndrome (HUS) is defined as the combination of microangiopathic hemolytic anemia (MAHA), acute renal failure, and thrombocytopenia. It occurs most often during outbreaks of diarrheal food poisoning with enteropathogenic strains of *Escherichia coli* such as O157:H7 and others that produce a shiga-like toxin.1 Less commonly, HUS may be associated with other infections, such as HIV and *Streptococcus pneumoniae*, various drugs such as calcineurin inhibitors and chemotherapeutic agents, and mucin-secreting adenocarcinomas.

Atypical HUS (aHUS) accounts for approximately 10% of all cases of HUS.2,3 The clinical manifestations are indistinguishable from diarrhea-associated HUS without the bloody diarrhea. Whereas diarrhea-associated HUS generally recovers after a period of supportive care, aHUS often recurs, may lead to end-stage renal failure and death in a high proportion of cases, and frequently recurs after renal transplantation. aHUS may be sporadic or familial. It usually presents in childhood but may occur at any age.

Endothelial injury leading to platelet aggregation and thrombotic microangiopathy (TMA), particularly in glomerular capillaries, underlies all forms of HUS. In diarrhea-associated HUS, endothelial damage is caused by the catalytic subunit of shiga-like toxin, which inhibits protein synthesis by cleaving 28S ribosomal RNA. In contrast, unregulated activation of complement accounts for at least 50% of cases of aHUS.2–4

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To explain the abnormalities underlying aHUS, a brief review of complement alternative pathway (AP) regulation is necessary. The AP is a positive-feedback loop that ticks over continuously under basal conditions, forming the AP C3 convertase C3bBb. This complex, composed of activated C3 and factor B (fB), attaches to cell surfaces through C3b, where it is capable of cleaving and activating additional C3, thereby generating C3bBbC3b. This in turn serves as a C5 convertase to produce C5a, a potent chemotaxin acting through the C5a receptor, and C5b, the initial step in assembly of C5b-9 known as the membrane attack complex.

Normally this process is held in check by several cell-associated and fluid-phase complement regulatory proteins (CRPs) that inhibit C3 activation and cause decay of the convertases. When a microbe enters the system, C3b binds to the microbial surface, which lacks CRPs, causing the process to accelerate and destroy the invader. At the same time, CRPs on host cells protect them from the activated complement cascade. Factor H (fH), factor I (fI), and membrane co-factor protein (MCP) are the predominant CRPs that are affected in aHUS. Cell-associated MCP and fluid-phase fH serve as co-factors for fI-mediated cleavage of C3b to iC3b. iC3b is incapable of binding fB and therefore cannot generate the C3 and C5 convertases.

aHUS may arise when there is deficiency, dysfunction, or autoantibody-mediated inhibition of one or more of the CRPs, resistance of C3b or fB to decay, or mutation of thrombomodulin, an endothelial glycoprotein with cytoprotective and anticoagulant properties. The disease may be quiescent for months or years, even in patients with homozygous mutations and complete deficiency, only to be triggered by an otherwise innocuous infection, drug exposure, or pregnancy. Mutations in fH, fI, and MCP cause inactivation or deficiency of the respective proteins. In addition, mutations in C3 and fB render the proteins resistant to fH and fI. Standard treatment involves plasma exchange to replace the deficient or defective fluid-phase CRPs or remove inhibitory antibodies; however, this is ineffective for MCP deficiency. Some patients with fH mutations may be cured by liver/kidney transplantation.

In addition to autoantibodies, mutations in fH are the most frequent abnormalities identified in aHUS, accounting for approximately 20 to 30% of cases. The gene lies in the regulation of complement gene cluster at 1q32 and codes for a protein produced by the liver and made up of 20 short consensus repeat domains called complement control protein modules. Modules in the C-terminal domain are responsible for binding to C3b and glycosaminoglycans and cell adhesion. Those in the N-terminus have co-factor activity.

Whereas discovery of mutations in fH and other CRPs in patients with aHUS provided evidence for their role in TMA, direct proof was lacking until 2007, when Pickering et al. described a transgenic mouse, Cfh−/−.FHΔ16−20, in which fH-deficient mice were crossed with transgenic mice carrying an fH mutation that had been identified in several patients with aHUS. The mutation produces a truncated fH lacking C-terminal short consensus repeat domains 16 to 20 that are responsible for surface adhesion. Remarkably, the mice developed a glomerular lesion that looks just like the TMA in patients with aHUS, including renal failure, thrombocytopenia, and MAHA. Although the truncated fH was able to regulate C3 activation in the plasma, it was unable to regulate C3b on the endothelial cells because it lacks the surface-binding domains.

In this issue of *JASN*, Goicoechea de Jorge et al. extend their findings in Cfh−/−.FHΔ16−20 mice to define further the role of complement in aHUS. They crossed Cfh−/−.FHΔ16−20 mice with mice deficient in C5 and observed complete protection from glomerular injury and MAHA, thereby documenting that activation of C5, presumably by unregulated production of the C5 convertase, is essential for the development of aHUS. As expected, C3 was deposited on the endothelial cells of both the C5-replete and C5-deficient Cfh−/−.FHΔ16−20 mice; however, it is noteworthy that the protected C5-deficient mice had less deposited C3. This suggests the possibility that, once injured, the endothelial cell membrane itself becomes an activating surface for additional C3b adhesion and further complement activation.

As in humans, the authors show that TMA is precipitated by an environmental stimulus in Cfh−/−.FHΔ16−20 mice that are still healthy, in this case by glomerular injury induced by accelerated nephrototoxic nephritis. Although the authors found glomerular endothelial deposition of C9 in the C5-replete but not in the C5-deficient mice, it has yet to be established whether the endothelial injury that results from C5 cleavage is due to C5a acting through the C5a receptor or to generation of C5b-9. Whatever the mechanism of C5-dependent injury, this study provides further preclinical support for the use of agents to neutralize C5, such as the mAb eculizumab, which has been shown to be effective in controlling acute exacerbations of aHUS in several case reports. It should also encourage clinicians faced with such cases to investigate them for complement regulatory disorders and enroll them in ongoing clinical trials (see ClinicalTrials.gov identifier NCT01194973).

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**REFERENCES**

chronic kidney disease (CKD), there is controversy over whether all patients need our attention. It has been argued, for example, that many patients with stage 3 or 4 CKD, especially the elderly, are not at increased risk for mortality and progression. Peralta et al. in this issue of JASN shed new light on this question. They studied the Multi-Ethnic Study of Atherosclerosis (MESA) and Cardiovascular Health Study (CHS) data sets to determine whether risk prediction for stages 3 and 4 CKD improves by measuring estimated GFR (eGFR) calculated with not only the creatinine Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation but also the cystatin C CKD-EPI equation. They show that risk for progression in patients with stage 3 CKD on the basis of the level of creatinine eGFR is not increased when cystatin C eGFR is \( >60 \text{ ml/min per 1.73 m}^2 \). Only in 50% of the cases in which cystatin C eGFR is also decreased is the risk for mortality, cardiovascular events, and heart failure elevated significantly. In addition, the risk for ESRD is only 2.6-fold elevated in patients with creatinine eGFR \(<60 \text{ ml/min per 1.73 m}^2 \) and normal cystatin C eGFR, whereas it is 23.8-fold elevated in cases in which cystatin C eGFR is also decreased. The additive value of cystatin C eGFR is also independent of albuminuria. Some issues should be considered in interpreting these findings.

First, why in this study does creatinine CKD-EPI eGFR not discriminate for increased vascular and mortality risk? The use of the creatinine CKD-EPI equation has been shown to result in reclassification of 30 to 40% of patients identified by the creatinine-based Modification of Diet in Renal Disease (MDRD) equation as having an eGFR between 30 and 60 ml/min per 1.73 m\(^2\). This reclassification was expected to result in better risk prediction of the smaller group of patients who have an eGFR of 30 to 60 ml/min per 1.73 m\(^2\) using the CKD-EPI formula. Data from the Atherosclerosis Risk in Communities (ARIC) study indeed showed the adjusted incidence ratio for mortality was 1.69 and 16.7 for ESRD in patients with a creatinine CKD-EPI eGFR between 30 and 60 ml/min per 1.73 m\(^2\) compared with a group with an eGFR between 90 and 120 ml/min per 1.73 m\(^2\). Similarly, in the Australian Diabetes, Obesity and Lifestyle (AusDiab) study, the adjusted hazards for all-cause mortality were 1.26 and 2.30 for the creatinine CKD-EPI eGFR groups 45 to 59 and 30 to 45 ml/min per 1.73 m\(^2\), respectively, compared with the group without CKD. These data seem contradictory to those in the current report from Peralta et al., in which patients with a creatinine CKD-EPI eGFR of <60 ml/min per 1.73 m\(^2\) do not have an increased mortality risk.

Several reasons may explain the seemingly contradictory findings, such as differences in cohort characteristics, for example, age, or in the choice of the reference category. With respect to age, it is important to note that the average age in the MESA and CHS...