Complement factor H and the hemolytic uremic syndrome

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Immune recognition is coupled to powerful proinflammatory effector pathways that must be tightly regulated. The ancient alternative pathway of complement activation is one such proinflammatory pathway. Genetic susceptibility factors have been identified in both regulators and activating components of the alternative pathway that are associated with thrombotic microangiopathies, glomerulonephritides, and chronic conditions featuring debris deposition. These observations indicate that excessive alternative pathway activation promotes thrombosis in the microvasculature and tissue damage during debris accumulation. Intriguingly, distinct genetic changes in factor H (FH), a key regulator of the alternative pathway, are associated with hemolytic uremic syndrome (HUS), membranoproliferative glomerulonephritis (dense deposit disease), or age-related macular degeneration (AMD). A mouse model of HUS designed to mirror human mutations in FH has now been developed, providing new understanding of the molecular pathogenesis of complement-related endothelial disorders.

Types of HUS

HUS and thrombotic thrombocytopenia purpura (TTP) constitute a group of diseases known as thrombotic microangiopathies in which organ damage results from platelet aggregation and fibrin plugs in small vessels. HUS, in particular, is characterized by microangiopathic hemolytic anemia (damage to red blood cells as they travel through narrowed capillaries), consumptive thrombocytopenia (exhaustion of platelets as they become enmeshed in platelet–fibrin thrombi), and microvascular glomerular thrombosis (formation of thrombi in the kidney). The thrombotic microangiopathy is particularly severe in the renal microvasculature and leads to acute renal failure. TTP shares these features, but neurologic dysfunction dominates the clinical presentation. Patients with TTP carry mutations in the metalloproteinase ADAMTS13 or have autoantibodies against this metalloproteinase,

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allowing for an etiologic distinction between TTP and HUS.

The most common form of HUS is associated with a diarrheal illness caused by infection with strains of *Escherichia coli* that produce Shiga-like toxins (Stx-1 and Stx-2) and hence is called Stx-HUS or diarrhea-positive HUS. Less common is atypical HUS (aHUS) in which there is no preceding diarrhea (nonenteropathic HUS). Precipitating factors commonly implicated in the pathogenesis of aHUS include infections, use of endothelial-damaging drugs, malignancies, transplantation, and pregnancy. These triggers can all cause endothelial cell activation and injury. Although there is some pathophysiologic overlap, Stx-HUS–induced pathology is predominant in the glomerulus, whereas the predominant pathology in aHUS involves the renal and interlobular arterioles. The prognosis of Stx-HUS is favorable, with the majority of patients recovering renal function, whereas in aHUS there is 25% acute mortality and most of the survivors develop end-stage renal disease.

Familial occurrence of aHUS has been recognized for many years (1). Inheritance was thought to be predominantly recessive, but it is now recognized that most families feature dominant inheritance with ∼50% penetrance. In 1998, Warwicker et al. (2) published the results of a linkage study in three families with aHUS. This pivotal study showed segregation of the disease to the q32 region of chromosome 1, which contains genes that regulate complement activation. A possible link between complement abnormalities and aHUS had also been recognized for many years. One study in particular showed that low levels of the complement protein C3 and glomerular deposition of C3 fragments were associated with disease (3). However, these clinical reports were not widely appreciated, as most patients had normal levels of circulating complement. And a role for innate immunity was not considered.

aHUS mutations have since been described in the genes encoding five complement proteins, including FH, membrane cofactor protein/CD46 (MCP), factor I (FI), factor B (FB), and C3 (4–8). Functional studies have shown that the mutations in the three regulators FH, FI, and MCP lead to loss of function and thus more complement activation, whereas the mutations in FB are gain of function. This has provided unequivocal evidence that complement dysregulation is involved in the pathology of aHUS. With this knowledge in hand, Pickering et al. (9) have now developed a faithful model of human aHUS in an FH-deficient mouse. The new mouse model, described in this issue (p. 1249), is sufficiently "human-like" so that key questions related to immune pathogenesis can now be addressed and potential therapeutic interventions assessed.

Alternative pathway of complement activation

The complement system (as one of our students recently informed us) is "a simple little proteolytic cascade when compared with cytokine biology and signal transduction pathways." It is an

ancient innate immune network of plasma proteins that began evolutionarily as a host defense system of hemolymph. The goal of the oldest cascade, the alternative pathway, is to rapidly coat invading microbes with large quantities of the opsonic complement fragment C3b (Fig. 1 A). This process is facilitated by a feedback or amplification loop that allows several million molecules of C3b to be deposited on bacteria within a few seconds. Complement activation also occurs, albeit usually in a more limited fashion, on altered self-tissue, such as on cells undergoing apoptosis and at sites of injury and infection. To prevent the accumulation of undesirable quantities of deposited C3b, the host synthesizes a family of regulatory proteins that inhibit the feedback loop both on cell surfaces and in plasma. These regulatory proteins are particularly designed to prevent C3 activation, and heterozygous mutations (haploinsufficiency) in these regulators predispose humans to aHUS. In individuals carrying these mutations, expression of even 50% of normal levels of these inhibitors is inadequate to prevent disease at times of injury and stress in the renal microvasculature.

FH

The first candidate gene in the regulators of complement activation (RCA) cluster that was linked to aHUS was FH,

a complement control protein (CCP) that protects host tissue from complement attack (2, 4). The surfaces of "foreign" particles, such as viruses and bacteria, are vulnerable to complement activity, as they lack FH and other complement regulatory proteins. FH regulates the feedback loop in three ways. It is a cofactor for the serine protease FI, which cleaves C3b. This cleavage reaction produces a fragment, iC3b, which has no hemolytic or amplification potential (Fig. 1 B). Second, FH both prevents the formation of and accelerates the disassociation of the alternative pathway C3 convertase, C3bBb. Finally, FH binds to polyanions on host cell surfaces and tissue matrices, such as basement membranes, thus blocking deposition of C3b. FH and other proteins within the RCA cluster share a common structural motif consisting of contiguous, homologous, independently folding modules called short consensus repeats or CCP modules. FH consists of 20 such repeats of ∼60 amino acids each that are held together by short 2– to 6–amino acid linkers.

Previous reports had shown a possible association between aHUS and reduced plasma concentrations of FH (10, 11). Mutations in the FH gene in aHUS patients have now been widely described. The interactive FH-HUS mutation database (http://www.FH-HUS.org) lists 71

disease-associated mutations (12). These are present in ∼30% of patients, including those with sporadic and familial forms of the disease. The majority are heterozygous missense mutations that cluster in the carboxyl-terminal exons and are associated with normal levels of circulating FH. The remaining mutations are mostly heterozygous deletions or missense mutations that result in either a truncated protein or impaired secretion of the protein and thus cause a 50% reduction in plasma levels of FH.

This clustering of missense mutations in the C-terminal region, which is crucial for FH binding to host surfaces, is remarkable. Functional and structural studies of mutants indicate that the reduced interaction between FH and cell surfaces leads to inefficient cofactor activity (12).

There are two existing animal models of total FH deficiency: the Norwegian Yorkshire pig (13) and a knockout mouse model (14). In both, the deficient animals develop membranoproliferative glomerulonephritis rather than HUS. This form of glomerulonephritis is similar to the disease seen in humans with homozygous FH deficiency in which dense deposits of C3 fragments accumulate in the glomerulus (15). This illness can present in a variety of ways, including asymptomatic urinary abnormalities, nephrotic syndrome, and chronic kidney disease. Patients with membranoproliferative glomerulonephritis also develop ocular lesions similar to those seen in AMD. A series of studies has shown that polymorphisms in the FH gene are associated with increased susceptibility to AMD (16–19). In particular, a polymorphism in exon 9 (rs1061107:1277T>C) that results in a tyrosine to histidine change (Y402H) in the seventh CCP, has been shown to be a susceptibility factor in all these studies.

A murine model of aHUS

In this issue, Pickering et al. describe a mouse model of aHUS in which the disease is remarkably similar to that seen in human patients. Taking advantage of the $FH^{-/-}$ mouse they had previously developed (14), the group produced a transgenic mouse in which the FH gene lacks

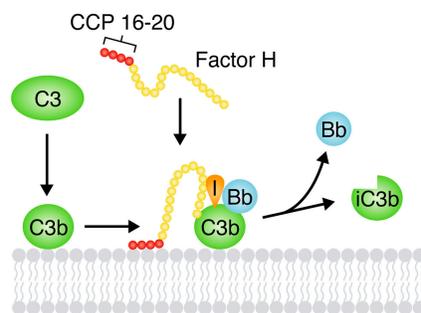**A Activation**
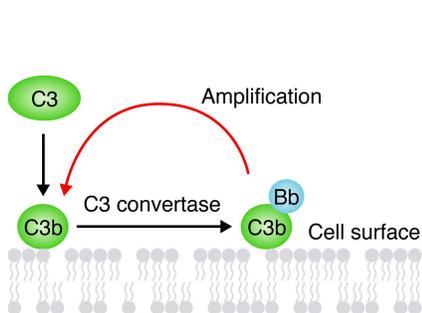
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Figure 1. Activation and inactivation of the alternative complement pathway. (A) C3b binds FB, which is then converted to Bb, by the serine protease factor D (not depicted). The resulting C3bBb is the alternative pathway C3 convertase that cleaves additional C3 to C3b, thus amplifying the process. Properdin stabilizes this convertase (not depicted). (B) This process is controlled by regulatory proteins such as FH. It decays Bb from C3b and is a cofactor for degradation of C3b to iC3b by the serine protease FI. FH attaches to surfaces through its heparin/anionic-binding sites in its carboxyl-terminus (CCPs 16–20) while the decay-accelerating activity and cofactor activity sites are at the amino terminus (CCPs 1–4).

the exons encoding the five C-terminal CCP modules. As noted above, in humans with FH–associated aHUS, a majority of the mutations occur in these C-terminal repeats and disrupt a heparin-binding site.

Homozygous FH-deficient mice have very low circulating levels of C3 and FB because these proteins are consumed as a result of excessive complement activation. Consequently, these mice have minimal complement-activating capacity. By introducing the C-terminal truncation of FH into the FH knockout animals, Pickering et al. restored the control of complement activation in the fluid phase. As a result, the mice did not develop glomerulonephritis, and their circulating levels of C3b and FB were partially restored. The combination of functional alternative pathway activation coupled with a deficiency in the capacity of FH to bind anionic targets led to spontaneous aHUS in mice homozygous for the transgene (Cfh$^{-/-}$.FHΔ16–20). Many parameters of disease in the mice, including the hematologic abnormalities and glomerular pathology, closely resembled human aHUS. Of note, however, the mice heterozygous for expression of the mutated FH (Cfh$^{+/-}$.FHΔ16–20; i.e., 50% of FH carrying the deletion of CCPs 16–20 and 50% WT FH), which most closely resembles the situation in humans, did not develop aHUS. As Pickering et al. comment, it is becoming apparent that aHUS in humans is not a straightforward monogenic disease, but rather a disease in which mutations may be found in more than one complement gene in an individual patient. Thus, such mutations must be considered to be predisposing rather than causative, as a triggering factor is necessary to initiate the disease (12). This murine model of aHUS affords a unique opportunity to resolve some of the conundrums of aHUS. The model will also facilitate testing of novel therapies, such as targeted complement inhibitors.

Other complement proteins implicated in HUS

In the second of the original three families reported by Warwicker et al., a mutation in MCP was identified (5). Screening of multiple cohorts established that ∼15% of patients with aHUS have a mutation (usually heterozygous) in the MCP gene (12). MCP is expressed by most cells and, like FH, acts as a cofactor for the inactivation of C3b. The activity of MCP is intrinsic, meaning that it only has cofactor activity for C3b when it is bound to the same cell (20). An analysis of the initial 25 MCP mutations in aHUS patients has recently been reported (21).

Mutations in FI, the protease required for C3b cofactor activity, also predispose to aHUS. Heterozygous changes in FI account for ∼5% of the known mutations in aHUS (6, 22). FI is a disulphide-linked, two-chain serine protease consisting of a 55-kD heavy chain and a catalytic 38-kD light chain. FI cleaves C3b but only if C3b is associated with a cofactor protein such as FH or MCP (Fig. 1 B; reference 22).

Mutations in FB were also recently described in two families (7). One mutation stabilized the alternative pathway C3 convertase by increasing the affinity of the interaction between FB and C3b. The other mutation made the C3bBb enzyme complex more resistant to decay. Both mutations resulted in increased activation of the alternative pathway. Mutations in C3 itself have also been described in a select group of aHUS patients with low C3 concentrations in their plasma but without mutations in FH, MCP, FI, or FB (8).

Immunopathogenesis of HUS

These analyses of aHUS-causing mutations in complement proteins establish several points of interest. First, although FH and MCP both harbor cofactor activity for FI, they do not have overlapping activity, as mutations in either protein predispose to disease in the renal microvasculature. It is also clear that half the normal levels of FH, MCP, or FI is not sufficient to protect the renal endothelium after injury (i.e., there is an evolutionary reason why these proteins are maintained at a certain level and not 50% less). In addition, anionic (heparin)-binding sites are required for FH to efficiently bind to damaged cells, exposed tissue matrices, and the basement membrane, and thus to prevent a thrombomicroangiopathy. It is noteworthy that the above insights came about by screening the human genome in multiplex families. The common theme that emerges relative to the specific functional deficiency in these complement regulators is a decrease in overall cofactor activity for C3b.

We hypothesize that Stx-HUS also follows the same sequence of events. The major difference between the Stx-HUS and aHUS may be secondary to the degree or type of damage to the endothelium required to cause disease. Less injury may be sufficient to cause aHUS in individuals with predisposing mutations, such as those in FH. A more severe degree of injury, such as that caused by Shiga toxins, may be required for HUS associated with *E. coli* epidemics. However, it is important to note that only 10–15% of patients in these epidemics develop HUS (23). Minor variations in the expression levels or functional activity of one or more members of the alternative pathway may be one explanation for this finding. We recently identified a young girl with a heterozygous mutation in MCP who developed Stx-HUS. *E. coli* O157:H7 was isolated from her stool, and Shiga toxin was identified in her blood and stool. The patient died 4 days after presentation. At autopsy, she had multiorgan failure secondary to a diffuse microthromboangiopathy. This informative case illustrates the interplay between a predisposing regulatory protein deficiency and the potency of the trigger in the pathogenesis of HUS.

We still have much to learn about the interplay among endothelial injury/stress, complement activation, and thrombosis in the microvasculature. The "Pickering model" of aHUS will allow many questions to be addressed. What, for example, is the nature of the damage induced by bacterial toxins and other triggers? Which parts of the complement activation cascade (opsonins, anaphylotoxins, membrane attack complex) induce thrombosis? How do the complement and clotting systems interact, and what is unique about the kidney microvasculature? This field of investigation was broken open by analyzing a few families using whole genome screens. Based on these findings, a mouse model has now been developed that can be used to

address outstanding questions about the pathogenesis and treatment of this complex human disease.

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