Editorial

Complement and disease: better no factor H than bad factor H

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Comment on: Ueda Y, Mohammed I, Song D, *et al*. Murine systemic thrombophilia and hemolytic uremic syndrome from a factor H point mutation. Blood 2017;129:1184-96.

Submitted Apr 21, 2017. Accepted for publication May 03, 2017.
doi: 10.21037/tcr.2017.05.24
View this article at: http://dx.doi.org/10.21037/tcr.2017.05.24

Within just a few minutes the complement system (1) can label a foreign cell that has invaded the bloodstream with millions of copies of C3b. Destruction or clearance of the C3b-coated invader quickly follows. C3b is the activated cleavage product of C3, an abundant serum protein. Numerous diseases have been linked to collateral damage from this aggressive process (2). Recently, Ueda *et al*. (3) presented a striking example of a mouse model, based on a single amino-acid replacement, in which uncontrolled C3b deposition on host tissue led to renal failure, stroke and retinopathy.

The abrupt and rapid expansion of C3b copy number is fuelled by multiple cycles of a positive-feedback loop (*Figure 1*) in which each nascent C3b molecule promotes conversion of C3 into more C3b molecules. While amplification of C3b can occur in the fluid phase, this is considered largely futile and consumes C3. More importantly, nascent C3b can transiently utilise its thioester group to bind covalently to nearby surfaces. The resulting clusters of surface-deposited C3b molecules trigger further steps in the complement cascade including release of the pro-inflammatory peptide C5a and formation of the membrane-attack complex (MAC).

The explosive nature of C3b amplification helps tackle pathogenic microbes that linger only briefly in the bloodstream. But the launch of a lag-free response to diverse threats is risky. It entails spontaneous generation of a trickle of C3b molecules that bind to any nearby surface thus priming it for C3b amplification. This indiscriminate and "always-on" mode of action necessitates the selective protection of self surfaces. Protection is achieved by a set of soluble and membrane-associated complement-regulating proteins. Most of these (4) work by suppressing C3b amplification on self surfaces. Soluble regulators also prevent futile fluid-phase C3 consumption. On damaged or "altered self", regulators permit controlled C3b deposition and some induce degradation of C3b to iC3b and C3dg, which promote non-inflammatory clearance. Regulators do not block C3b amplification on "nonself", e.g., microbial surfaces, indeed soluble regulators resist hijack by microbes.

Prominent amongst soluble regulators of spontaneous C3b formation and amplification is the 155-KDa glycoprotein, factor H (FH) (5). While the N-terminal region of FH is responsible for shutting down C3b amplification regardless of context, the C-terminal region ensures FH focuses its regulatory efforts on self surfaces rather than nonself ones (6,7). Ueda *et al*. graphically demonstrated the severity of disease phenotype that can arise when FH is mutated in a way that permits C3b clusters to form on host-tissue surfaces.

Some cancer cells are recognised as nonself or altered self and are susceptible to complement-mediated attack or clearance (8). To avoid this, cancer cells may overexpress FH and other complement regulators (9,10) or (like some bacteria) may attempt capture of circulating FH for their own protection (11). FH is a possible diagnostic biomarker and potential target for anticancer therapy (12). Autoantibodies from lung cancer patients that targeted the C-terminal region of FH inhibited its binding to lung tumour cells, increased C3b deposition and caused complement-dependent tumour cell lysis (13). This points to a potential anti-FH antibody-based therapy.

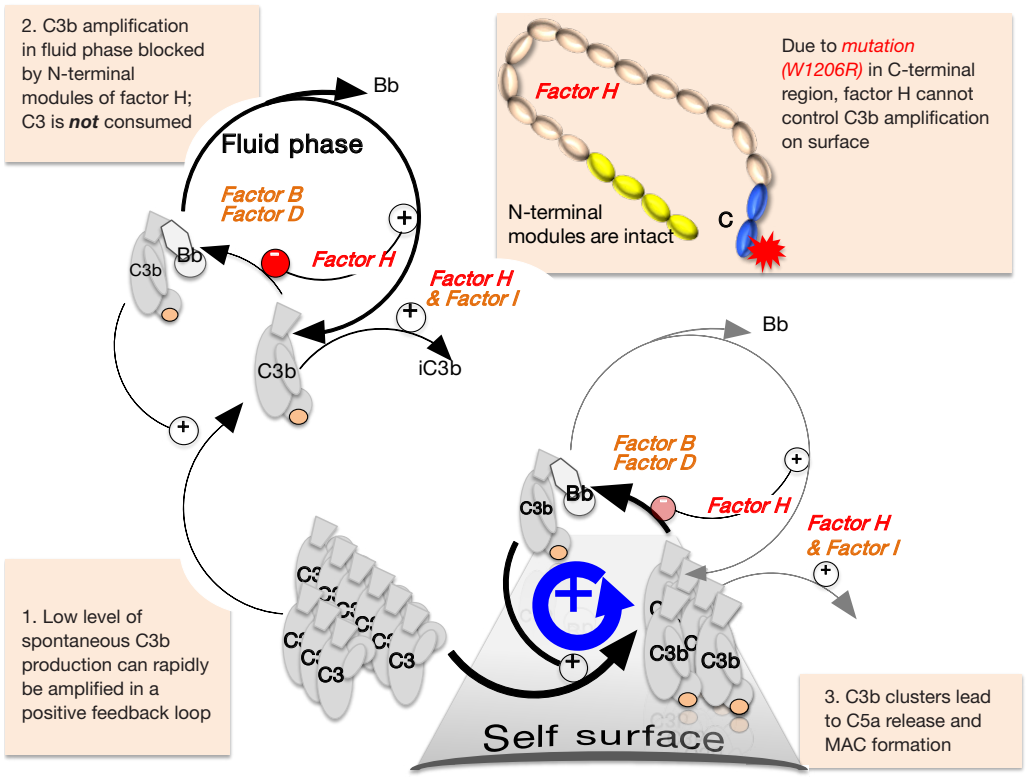

Figure 1 A murine factor H mutation with a severe phenotype. The W1206R mutation in the C-terminal module of murine factor H leaves suppression of C3 consumption in fluid phase unaffected, but perturbs control of the positive-feedback loop on surfaces. Factor B binds to C3b to form C3bB that is then cleaved by factor D to form C3bBb. This is a "C3 convertase" able to cleave C3 to generate additional C3b molecules. Factor I, with factor H as cofactor, cleaves C3b to iC3b. Factor H both competes for binding of factor B and accelerates the decay of C3bBb.

Conversely, an inflammatory microenvironment favours the development of many tumours. In such cases, cancer cells may be disadvantaged rather than advantaged by the recruitment of FH (14) since its anti-inflammatory properties may suppress tumour growth

FH has 20 modules (*Figure 1*) and 40 disulfides so it is difficult to produce recombinantly (15). But it is abundant in blood. Its roles are highlighted by the consequences of its absence in FH-knockout mice (16). In $FH^{-/-}$ mice, high levels of C3b, iC3b and C3dg are deposited in glomeruli, and serum C3 levels are depleted due to unrestrained fluid-phase conversion of C3 to C3b. This phenotype resembles C3 glomerulopathy (C3G), a human kidney disease seen in individuals whose FH has mutations, predominantly in its N-terminal modules. People with certain mutations in FH C-terminal modules (17), or mice in which the FH C-terminal region has been deleted (18), are predisposed to atypical haemolytic uraemic syndrome, a different kidney disease that is a form of thrombotic microangiopathy.

Damage arising from a complete absence of FH is moderated by fluid-phase (unregulated) consumption of C3. Conversely, FH with an intact N terminus and a mutated C terminus could be more injurious given that fluid-phase levels of C3 (the precursor of C3b) would be retained, but self surfaces would have lost part of their protection against C3b amplification (*Figure 1*). Many disease-related mutations cluster in CCPs 19 and 20. In heterozygous individuals, the phenotypic consequences of such a mutation on one allele may be masked by the presence of the "good" allele. And there are other membrane-associated regulators that might be expected to compensate for the loss of FH. Ueda *et al.* have now revealed the devastating consequences of homozygosity for a C-terminal mutation in FH.

To design their model, they speculated that the aHUS-linked FH mutation W1183R (19) in humans could be recapitulated in murine FH since this Trp residue

is conserved between mice and men. In HEK293 cells they made murine CFH with the equivalent mutation, W1206R. They confirmed that this version of FH regulates complement activation in the fluid phase (because it has an intact N terminus) but does not interact with self-surfaces. Then they generated mutant mice, using homologous recombination in C57BL/6J embryonic stem cells. The resultant homozygous animals, carrying the mutation W1206R ($FH^{R/R}$), were used to assess the consequences.

The severity of the outcome exceeded expectations. At 3–4 weeks of age, the $FH^{R/R}$ mice were smaller and thinner than $FH^{R/W}$ or $FH^{W/W}$ littermates. $FH^{R/R}$ mice carrying this mutation did not just develop renal thrombotic microangiopathy but also developed systemic thrombophilia involving large blood vessels in the brain, liver, lung, spleen and kidney. Nearly one third of the tested mice displayed symptoms of stroke and ischemic retinopathy. The onset of organ thrombosis and renal injury occurred within the first week of life, sudden death was observed on numerous occasions, and just under half of the $FH^{R/R}$ animals (but none of the $FH^{W/R}$ animals) died within 30 weeks.

Presumably in the absence of FH protection on host surfaces, C3b amplification is not fully controlled, despite the presence of other membrane-associated complement regulators. The balance is tipped in favor of amplification, clusters of C3b form and the consequent C5a production and MAC formation causes injury to vascular endothelial and smooth muscle cells, creating a prothrombotic state of the vessel wall. MAC and C5a might also directly activate platelets and neutrophils or monocytes, respectively, leading to release of tissue factor from the stimulated leukocytes, platelet and leukocyte aggregation, and clot formation in both capillaries and large blood vessels.

Severe retinal injury was seen in most $FH^{R/R}$ mice. While there is no known connection between retinopathy and the W1183R mutation in human FH, FH dysfunction has been strongly linked to the pathogenesis of AMD. For example R1210C, at the C-terminus of FH, originally found in some aHUS patients, is a highly penetrant mutation for dry AMD (20). Ueda *et al*. did not characterize the specific pathology of retinopathy in these animals so could not demonstrate direct complement-mediated attack on retinal cells. The observed retinal injury could be secondary to vascular occlusion.

To establish that the observed phenotype was indeed a consequence of uncontrolled C3b deposition, they crossed their $FH^{R/R}$ mice with mice in which either C3 or factor D (FD; another key protein in the positive-feedback loop for C3b amplification, Figure 1) had been knocked out. The $FH^{R/R}C3^{-/-}$ and $FH^{R/R}FD^{-/-}$ mice had none of the symptoms displayed by their $FH^{R/R}$ littermates. They showed normal kidney function, platelet count and haemoglobin levels, their livers were thrombus free, and they had normal-looking retina and normal retinal blood flow. They lived normally and did not develop stroke symptoms.

The rarity of disease-linked mutations in the N-terminal and C-terminal regions of FH found in C3G and aHUS patients ensure that there are few homozygous cases. Nonetheless, by impairing FH activity on self surface while leaving its fluid-phase regulatory efficacy intact, the authors have revealed a surprising role of FH in preventing macrovascular thrombosis as well as thrombotic microangiopathy and renal disease. The data suggest that dysfunction or variation of FH activity on the cell surface could be implicated in human thrombotic disorders such as stroke and retinal arterial or venous occlusion, in addition to primary or secondary thrombotic microangiopathy. They also suggest that targeting the C terminus with a blocking antibody in cancer therapy could have unwanted consequences.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Peipei Xu (Department of Hematology, The Affiliated Drum Tower Hospital of Nanjing, University Medical School, Nanjing, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2017.05.24). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Barlow PN. Complement and disease: better no factor H than bad factor H. Transl Cancer Res 2017;6(Suppl 3):S606-S609. doi: 10.21037/tcr.2017.05.24