A mouse model of non-Shiga toxin-associated haemolytic uraemic syndrome*

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Introduction

Impaired control of the complement system activation due to mutations in complement factor H (CFH) has been described in two apparently unrelated human diseases, membranoproliferative glomerulonephritis type II (MPGN2) and non-Shiga toxin-associated haemolytic uraemic syndrome (non-Stx-HUS). Mouse models of these diseases have been developed by Pickering et al., by knocking-out Cfh gene (MPGN2) and by subsequently transferring a mutated Cfh gene in the Cfh−/− background (non-Stx-HUS). The data obtained from the two models provided precious information to clarify the mechanisms that cause the disparate phenotypes underlying CFH genetic defect.

MPGN2

MPGN2 is a rare cause of chronic nephritis characterized by the presence of dense deposits within the glomerular basement membrane (GBM), capillary wall thickening, mesangial cell proliferation and glomerular fibrosis [1]. The disease is associated with multiple complement abnormalities, including intense deposition of C3c in GBM deposits and persistent reduction of C3 serum levels [1,2].

Non-Stx-HUS

Non-Shiga toxin-associated haemolytic uraemic syndrome (non-Stx-HUS) is a rare disease with manifestations of haemolytic anaemia, thrombocytopenia and renal failure. The clinical outcome is unfavourable, with up to 50% of patients progressing to end-stage renal failure and 25% dying during the acute phase [3]. Persistently and remarkably depressed levels of the third component (C3) of the complement system have been documented in some patients with non-Stx-HUS [4–6] suggesting the presence of an inherited defect causing hyperactivation of the complement cascade.

CFH

CFH plays a key role in the regulation of the alternative pathway of complement (Figure 1). It serves as a cofactor for the C3b-cleaving enzyme, factor I (CFI), in the degradation of newly formed C3b molecules and controls decay, formation and stability of the C3b convertase C3bBb. CFH consists of 20 homologous short consensus repeats (SCRs) (Figure 2, panel A). The complement regulatory domains needed to prevent fluid phase alternative pathway amplification have been localized within the N-terminal SCR1−4 [7]. The inactivation of surface-bound C3b is dependent on the binding of the C-terminal domain of CFH to polyanionic molecules that increases CFH affinity for C3b and exposes its complement regulatory N-terminal domain (Figure 2, panel B). The C-terminal domains contain two C3b binding sites, located in SCR12−14 and SCR19−20 and three polyanion-binding sites, located in SCR7, SCR13 and SCR19−20 [8]. However, the C3b and the polyanion-binding sites located in SCR19−20 are the only indispensable for CFH to inactivate surface-bound C3b, since deletion of this portion of the molecule causes loss of CFH capability to prevent complemen activation on sheep erythrocytes [9].

Six mutations in CFH in MPGN2 patients have been described [10] that determine undetectable or markedly reduced protein levels (Figure 2, panel A). Out of seven patients, five are homozygous or compound heterozygous. Three mutations affect framework cysteine residues and block CFH secretion and intracellular catabolism [11]. CFH mutations in non-Stx-HUS patients are generally heterozygous and cause either single amino acid changes or premature translation interruptions, mainly clustering...
in the C-terminus domains (Figure 2, panel A) and are commonly associated with normal CFH plasma levels [10,12]. Expression and functional studies demonstrated that CFH proteins carrying HUS-associated mutations have a severely reduced capability to interact with polyanions and with surface-bound C3b [8], which results in a lower density of mutant CFH molecules bound to endothelial cell surface and a diminished complement regulatory activity on the cell membrane [8]. In contrast, these mutants have a normal capacity to control activation of the complement in plasma, as indicated by data that they retain a normal cofactor activity in the proteolysis of fluid-phase C3b [8] (Figure 2, panel C).

These genetic data support the hypothesis that distinct functional alterations in CFH are critical in conferring the specific phenotype of non-Stx-HUS and MPGN2.

What did we learn from mouse models of MPGN2 and HUS?

CFH knock-out mice (Cfh−/−) were generated by Pickering et al. [13], and were viable and fertile under pathogen-free conditions. CFH was absent and C3 and CFB levels were markedly reduced. At sacrifice (8 months) mice showed histological evidence of MPGN.

CFB knock-out mice (Bf−/−) [14] were viable and showed no gross phenotypic abnormalities but were unable to activate the alternative pathway of complement.

In the double KO mice Cfh−/−Bf−/− obtained by Pickering et al. [13], C3 levels were normal, renal function and histology did not differ from those of wild type animals. The blockade of C3 activation in vivo, due to Cfb deletion in those mice, prevented the development of MPGN. The absolute requirement for uncontrolled C3 activation in the pathogenesis of MPGN in Cfh−/− is also supported by the chronology of renal lesions (deposition of C3 in the glomerulus precedes the appearance of subendothelial electron-dense deposits).

In the paper discussed here [15], the authors generated transgenic mice on the Cfh−/− background expressing a mouse CFH protein that lacked the terminal 5 SCR domains (FHΔ16–20) [15], the equivalent mouse location of the majority of non-Stx-HUS associated mutations [12]. The Cfh−/−.FHΔ16–20 mice spontaneously developed HUS, and haematologic parameters and renal pathology closely resembled human non-Stx-HUS. Of note, the spontaneous pathology in those mice targeted the renal vasculature, suggesting that there are unique anatomical and/or physiological properties of this endothelial bed that render it particularly sensitive to complement mediated damage.

The data obtained clearly demonstrate that plasma C3 regulation was retained, while regulation of C3 activation on renal endothelium was defective. Thus, non-Stx-HUS develops when a combination of effective plasma C3 regulation and defective regulation on renal endothelium is present simultaneously (Figure 2, panels B and C).

At variance with non-Stx-HUS in humans, that is generally associated with CFH heterozygous mutations, Cfh+/−.FHΔ16–20 mice do not develop the disease. Multiple genetic defects have been reported in few non-Stx-HUS patients, and conditions that trigger complement activation either directly (bacterial and viral infections) or indirectly by causing endothelial insult (drugs, certain systemic diseases) may precipitate an acute event on the predisposed genetic background [12].

It is possible also that Cfh+/−.FHΔ16–20 mice may only develop the disease after an additional insult. For this reason Cfh+/−.FHΔ16–20 mice could be a useful model to study the effect of various triggers, although interspecies differences in the regulation of C3 on cell surfaces by CFH and other complement regulators may also be relevant.

Clinical implications

The key observation of the report by Pickering et al. is that a combination of effective plasma C3 regulation and defective regulation on renal endothelium is required for non-Stx-HUS to develop. This scientific achievement implicates important aspects from a therapeutic point of view. New therapeutic agents should be developed that can restore C3 regulation on cell surfaces. In fact the
Fig. 2. Panel A: CFH functional domains and variants described in non-Stx-HUS and MPGN2. Panel B: Proposed model for CFH mechanism of action. After viral or bacterial infection or endothelial insult, complement is activated and C3b is formed. In the presence of normal CFH, C3b is rapidly inactivated to inactive C3b (iC3b). CFH in the circulation binds fluid-phase C3b and favours its degradation by factor I (CFI). In addition, it binds to polyanionic proteoglycans that are present on endothelial cell surface, where, because of its high affinity for C3b, it entraps fluid-phase C3b, thus preventing its deposition on host surfaces and its binding with factor B (CFB) to form the C3 convertase complex (C3bBb). Panel C: Proposed consequences of CFH mutations found in patients with HUS. Mutant CFH has a normal cofactor activity in fluid phase. However, the mutations affect the polyanion interaction site at the C-terminus of CFH so that it shows reduced binding to proteoglycans on endothelial cell surface. This results in more C3b reaching the endothelial cell surface. In addition, C3b deposited on cell surface is not degraded and forms the C3 convertase of the alternative pathway of complement that further cleaves C3.

Restoration of fluid phase regulation alone may be deleterious by increasing circulating plasma levels of C3. CFH is mainly produced by the liver; thus, a kidney transplant in CFH mutation carriers is contraindicated, while combined liver and kidney transplantation should represent the definitive treatment. The mouse model could be a proper instrument to better evaluate the safety of this procedure that has been used in few cases and produced mixed results [16–18].

In conclusion, the paper by Pickering et al. adds a plug in the comprehension of the pathogenesis of non-Stx-HUS and of MPGN, suggesting a clear genotype–phenotype relationship.

**Take home message**

Complete deficiency of CFH is associated with MPGN2, while non-Stx-HUS is associated with heterozygous CFH mutations that do not affect CFH circulating levels. A combination of effective plasma C3 regulation and defective regulation on renal endothelium is required for non-Stx-HUS to develop.

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