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An extended mini-complement factor H molecule ameliorates experimental C3 glomerulopathy

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Abnormal regulation of the complement alternative pathway is associated with C3 glomerulopathy. Complement factor H is the main plasma regulator of the alternative pathway and consists of 20 short consensus repeat (SCR) domains. Although recombinant full-length factor H represents a logical treatment for C3 glomerulopathy, its production has proved challenging. We and others have designed recombinant mini-factor H proteins in which 'non-essential' SCR domains have been removed. Here, we report the *in vitro* and *in vivo* effects of a mini-complement factor H protein, $FH^{1-5\wedge18-20}$, using the unique factor H–deficient (*Cfh* − / −) mouse model of C3 glomerulopathy. $FH^{1-5\wedge18-20}$ is comprised of the key complement regulatory domains (SCRs 1–5) linked to the surface recognition domains (SCRs 18–20). Intraperitoneal injection of $FH^{1-5\wedge18-20}$ in *Cfh* − / − mice reduced abnormal glomerular C3 deposition, similar to full-length factor H. Systemic effects on plasma alternative pathway control were comparatively modest, in association with a short half-life. Thus, $FH^{1-5\wedge18-20}$ is a potential therapeutic agent for C3 glomerulopathy and other renal conditions with alternative pathway-mediated tissue injury.

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Factor H (FH, 150 kDa) is the key regulator of complement activation in plasma.[1,2] FH inhibits the C3b amplification loop by preventing formation and promoting dissociation of the alternative pathway (AP) C3 convertase, and by acting as a cofactor for factor I (FI)–mediated C3b cleavage.[1,3] It comprises 20 short consensus repeat (SCR) domains, with amino (N)-terminal SCRs 1–4 accounting for its AP regulatory functions. FH is targeted to specific tissues via two binding domains that recognize different heparan sulfate species.[4] The principle binding site for heparan sulfate species of the retina is located in SCR 7, whereas SCRs 19–20 target glomerular heparan sulfate.[5] Indeed, C-terminal SCRs 19–20 are critical for interaction with tissue-bound C3b and cell surface polyanions, enabling FH to regulate the AP on host cell surfaces.[2,6] Uncontrolled C3 activation via the AP because of deficiency or dysfunction of FH is associated with the development of C3 glomerulopathy (C3G).[7] The pathological hallmark of C3G is predominant C3 accumulation within the glomerulus, leading to end-stage kidney disease within 10 years of diagnosis in ~40% of patients.[8] Currently, there is no proven treatment for C3G, although a small prospective trial and several case reports suggest that the C5 inhibitor eculizumab may be beneficial in some patients.[9–11]

Mice with targeted homozygous deficiency of murine FH (*Cfh* − / −) demonstrate low plasma C3 levels and a linear pattern of C3 staining in the glomerulus, and eventually develop membranoproliferative glomerulonephritis.[12] Murine experimental C3G thus provides a useful model for studying the pathogenetic mechanisms and response to novel therapeutics of C3G. Injection of human FH or murine Cfh has been shown to restore plasma C3 levels and reduce glomerular C3 deposition in *Cfh* − / − mice at 24 h.[13,14] Accordingly, recombinant FH has been proposed as a logical treatment for establishing physiological AP control and halting disease progression in patients with C3G. However, because of the size and complexity of the FH protein, production of therapeutic quantities represents a significant challenge.[15–17] Here, we describe the generation and successful administration in *Cfh* − / − mice of a mini-FH protein ($FH^{1-5\wedge18-20}$), which is comprised of the key functional domains of FH. $FH^{1-5\wedge18-20}$ reduced glomerular C3 reactivity similar to full-length FH while also partially restoring plasma

C3 levels. These data indicate a plausible therapeutic role for $FH^{1-5\wedge18-20}$ in patients with C3G and other disorders of complement regulation.

RESULTS

Generation of FH^{1-5} and the Newcastle mini-FH ($FH^{1-5\wedge18-20}$) proteins

For our mini-FH construct, we included the first five SCRs of the N-terminal region of FH, as we and others have found that SCRs 1–5 of FH has improved function over SCRs 1–4 (Supplementary Figure S1 online; Huang *et al.*,[18] Cheng *et al.*,[19] and Gordon *et al.*[20]); SCR 5 is also the target of monoclonal antibody OX-24,[21] allowing an additional means to purify/identify the construct. We included SCR 18 at the C terminus to provide spacing to the construct and included a hexa-histidine tag as the primary means of purification, as well as providing additional flexibility/spacing between SCRs 5 and 18. $FH^{1-5\wedge18-20}$ and FH^{1-5} were expressed in Chinese hamster ovary cells and yielded 2 and 4 mg/l, respectively. After two-step purification, sodium dodecyl sulfate-polyacrylamide gel electrophoresis confirmed the purity of both FH^{1-5} and $FH^{1-5\wedge18-20}$, and that they migrated at their predicted molecular weight of ~36 and 59 kDa, respectively (Figure 1a). To assess the binding of $FH^{1-5\wedge18-20}$ to key ligands, microtiter plates were coated with C3b or heparin (a model polyanion) and binding of equimolar amounts of FH, FH^{1-5}, and $FH^{1-5\wedge18-20}$ measured. All three FH proteins bound to C3b, whereas only FH and $FH^{1-5\wedge18-20}$ bound heparin as detected by a polyclonal anti-FH (Figure 1b and c). FH and $FH^{1-5\wedge18-20}$ bound to C3b or heparin in an equivalent manner and could be inhibited by specific monoclonal antibody, confirming the specificity of the interaction in this assay. In light of recent findings regarding deregulation of FH function by the FHR proteins[22] and to establish if $FH^{1-5\wedge18-20}$ was also susceptible to deregulation, we carried out a C3b-binding/competition assay using equimolar amounts of FH, FH^{1-5}, and $FH^{1-5\wedge18-20}$ and increasing doses of recombinant FHR1 or FHR5. In this assay, $FH^{1-5\wedge18-20}$ was significantly more resistant to deregulation compared with FH at all concentrations of FHR (Figure 1d and e) but was still deregulated at the highest FHR doses (1.8 and 0.6μmol/l, respectively).

Fluid-phase regulatory capacity of recombinant $FH^{1-5\wedge18-20}$ is comparable to serum-derived FH

FI requires a cofactor such as FH to mediate cleavage of the C3b α-chain. The ability of $FH^{1-5\wedge18-20}$ to serve as a cofactor for FI was initially tested in a standard fluid-phase cofactor assay[23] and then in an SRBC (sheep red blood cells) assay.[24] C3b, FI, and a cofactor (FH, FH^{1-5}, or $FH^{1-5\wedge18-20}$) were incubated at varying equimolar concentrations. Encouragingly, $FH^{1-5\wedge18-20}$ performed significantly better than FH^{1-5} and was consistently more potent than serum-derived FH in the protection of C3b-coated SRBCs from lysis in this assay (Figure 2a). However, while comparable to FH, $FH^{1-5\wedge18-20}$ was not superior in the classical fluid-phase assays (Supplementary Figure S2 online). We also measured the decay-accelerating activity of FH and the recombinant constructs using C3b-coated SRBCs; again, FH and $FH^{1-5\wedge18-20}$ demonstrated 10-fold greater ability to decay the C3b convertase on SRBCs compared with FH^{1-5}. However, in this assay $FH^{1-5\wedge18-20}$ decay-accelerating activity function was indistinguishable from that of FH (Figure 2b). Overall, these data suggest that $FH^{1-5\wedge18-20}$ has maintained the complement regulatory function of FH.

$FH^{1-5\wedge18-20}$ is highly efficient in protecting SRBCs from complement attack by aHUS patient or FH functionally depleted serum

SRBCs are normally resistant to AP-driven complement attack because FH readily binds to their surface via its C terminus. This fact has been exploited by Sanchez-Corral *et al.*[25] who demonstrated that serum from patients with mutations in FH that affected the C terminus would readily lyse SRBCs. We have previously used this assay to confirm a defect in complement regulation in a patient with an FH hybrid gene.[26] Addition of FH or $FH^{1-5\wedge18-20}$ to serum from an aHUS (atypical hemolytic uremic syndrome) patient with C-terminus mutation was highly effective in blocking SRBC lysis (Figure 3b), confirming that increasing FH levels is sufficient to reverse an inherent regulatory defect in patient serum, with $FH^{1-5\wedge18-20}$ being equivalent to an equimolar concentration of FH. Using OX-24 to block FH function in the normal serum, which prevents FH binding to C3b on the erythrocyte surface,[27] we were able to show that functional loss of FH regulatory function by antibody blockade of the N terminus of FH can also be reversed by the addition of FH or $FH^{1-5\wedge18-20}$ (Figure 3c). The data indicate, in these cell-based assays, that $FH^{1-5\wedge18-20}$ is marginally more effective compared with FH in complement regulatory function in patient serum and may be useful for treatment of patients with either defective FH or antibodies that block FH function.

$FH^{1-5\wedge18-20}$ reduces glomerular C3 staining in *Cfh* −/− mice at doses that incompletely restore plasma C3 levels

We assessed the ability of $FH^{1-5\wedge18-20}$ to influence plasma and glomerular C3 *in vivo* by administering the protein to *Cfh* −/− mice (Figure 4a). After a single intraperitoneal 12 nmol dose of either $FH^{1-5\wedge18-20}$ or FH^{1-5}, plasma C3 levels significantly increased at 2 and 6 h after injection reaching ~20% of wild-type levels at 6 h (Figure 4a). Both injected proteins were detectable in plasma at 2 h after injection but absent or barely detectable at the 6 h time point (Figure 4b). In contrast, after a single injection of 3 nmol of serum-derived FH (the dose used successfully by Fakhouri *et al.*[14]), the injected FH was detectable at comparable concentrations 2 and 6 h after injection, and plasma C3 increased to ~60% of wild-type levels at the 6 h time point (Figure 4a and b). Glomerular C3 immunostaining fluorescent intensity was significantly reduced 6 h after injection of $FH^{1-5\wedge18-20}$ to a comparable degree to that seen following FH (Figure 4c). No significant reduction in glomerular C3 staining was seen in