Treatment with human complement factor H rapidly reverses renal complement deposition in factor H-deficient mice

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Total deficiency of complement factor H (CFH) is associated with dense deposit disease and atypical hemolytic uremic syndrome. CFH is the major regulator of the alternative pathway of complement activation and its complete deficiency results in uncontrolled C3 activation through this pathway and secondary C3 deficiency. Plasma infusion, as a source of CFH, has been used with variable success to treat renal disease associated with its deficiency. However, the risks of volume and protein overload limit this therapeutic approach. In this study, we investigated the efficacy of a purified human CFH (hCFH) preparation in *Cfh*-gene knockout mice. These mice spontaneously develop both secondary plasma C3 deficiency and a renal abnormality characterized by massive accumulation of C3 along the glomerular basement membrane. The renal lesion is analogous to human dense deposit disease. Treatment of knockout mice with hCFH resulted in rapid normalization of plasma C3 levels and resolution of the glomerular basement membrane C3 deposition. Long-term treatment of mice with hCFH was not possible because of the development of an immune response against hCFH. Hence, we suggest that hCFH can be an effective alternative therapy to plasma infusions in patients with renal disease associated with CFH deficiency.

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Complete deficiency of complement factor H (CFH) is associated with dense deposit disease (DDD) and atypical hemolytic uremic syndrome (aHUS). DDD is characterized by the presence of intra-membranous electron-dense transformation of the glomerular basement membrane (GBM).[1] The light microscopic features of DDD are heterogeneous but include membranoproliferative inflammation. DDD is associated with uncontrolled activation of the complement alternative pathway (AP).[1] The key AP regulator is the plasma protein CFH. Complete genetic deficiency of CFH, results in uncontrolled AP activation and severe secondary C3 deficiency (reviewed in Pickering and Cook[2]). Complete deficiency is also associated with DDD.[3] DDD is also associated with other causes of AP dysregulation, including autoantibodies that inhibit CFH function,[4,5] dysfunctional C3 molecules,[6,7] and autoantibodies that stabilize the AP C3-cleaving enzyme complex (C3 nephritic factors).[8,9] Animal models have reinforced the importance of uncontrolled AP activation in DDD. Gene-targeted CFH-deficient mice ($Cfh^{-/-}$) spontaneously develop low plasma C3 levels and deposition of C3 along the murine GBM.[10–12] This results in electron-dense GBM change and glomerular inflammation. A spontaneous mutation resulted in a breed of CFH-deficient pigs that develop both low plasma C3 levels and membranoproliferative glomerulonephritis (MPGN).[13–17]

At present, there is no definitive therapy for DDD.[1] The condition is associated with end-stage renal failure in a significant proportion of patients and has a very high recurrence rate in renal transplants.[1,18] Studies in $Cfh^{-/-}$ mice showed that activation of the AP is essential for the renal disease to develop. Thus, $Cfh^{-/-}$ mice that are rendered deficient in the AP activation protein factor B, do not develop renal disease (reviewed in Pickering and Cook[2]). Sequential histological studies in both pig and mouse models showed that the initial renal histological abnormality was the deposition of C3 along the GBM.[10,16] These observations suggest that key properties of an effective therapy in DDD would include the ability to regulate AP activation and to facilitate the removal of C3 along the GBM. An obvious

definitive therapeutic in CFH deficiency is CFH itself. Previously, we administered purified mouse CFH (mCFH) to $Cfh^{-/-}$ animals.[19] This resulted in the removal of C3 along the GBM, with concomitant increase in plasma C3 levels over a relatively rapid time frame of 24 h. In this study, we examined the efficacy of human CFH (hCFH) in the $Cfh^{-/-}$ mouse model. Our data show that administration of hCFH to $Cfh^{-/-}$ mice resulted in rapid normalization of plasma C3 levels and resolution of GBM C3 deposits. These data suggest that hCFH will be an effective therapy in individuals with DDD associated with CFH dysfunction. Long-term assessment of hCFH in mice was not possible because of the development of an immune response against hCFH.

RESULTS

Administration of hCFH restored plasma AP regulation in $Cfh^{-/-}$ mice

$Cfh^{-/-}$ mice spontaneously develop secondary C3 deficiency.[10] Hence, we first tested the ability of hCFH to restore AP regulation by measuring plasma C3 levels after hCFH administration. A single injection of 0.5 mg hCFH intraperitoneally increased plasma C3 levels at 2 h, peaking at 24 h, at which time the plasma C3 levels were comparable with normal wild-type C3 levels (Figure 1a). In injected $Cfh^{-/-}$ mice, plasma C3 levels remained normal at 48 h decreasing to ~50% of normal levels by 72 h and dropping to baseline values by 96 h (Figure 1b). We next determined the activation status of the plasma C3 by performing western blot analysis for C3 under reducing conditions using serum obtained from hCFH-injected mice. This enabled us to differentiate between intact C3 and its activation products. Intact C3 α-chain and β-chain were detectable in hCFH-injected $Cfh^{-/-}$ mice (Figure 1c). Intact C3 α-chain, absent in phosphate-buffered saline (PBS)-injected $Cfh^{-/-}$ mice, was detected as early as 2 h after administration of hCFH (Figure 1c). These data showed that, in this heterologous *in vivo* system (hCFH, mouse C3, and mouse CFI), hCFH was able to restore plasma AP regulation in $Cfh^{-/-}$ mice.

The serum half-life of hCFH was influenced by the degree of C3 activation

We measured hCFH levels by enzyme-linked immunosorbent assay (ELISA) before and at 24, 48, 72, 96, and 192 h (day 8) after the intraperitoneal administration of 0.5 mg hCFH (Figure 1d). In $Cfh^{-/-}$ mice ($n=4$), serum hCFH levels were highest in the 24 h samples (median 75.3 mg/l, range 63.6–85.9) and decreased to very low levels by 96 h (median 5.6 mg/l, range 2.9–6.7). hCFH was undetectable by day 8. In parallel, we administered an identical dose of hCFH to C3-deficient $Cfh^{-/-}$ mice ($Cfh^{-/-}.C3^{-/-}$, $n=3$). At each time point, the median levels of hCFH were higher in $Cfh^{-/-}.C3^{-/-}$ mice than in mice deficient in CFH alone. At 96 h, significant levels of hCFH were still detectable in $Cfh^{-/-}.C3^{-/-}$ mice (31.6 mg/l, range 28.5–31.9, $n=3$) compared with very low levels in $Cfh^{-/-}$ animals (median 5.6 mg/l, $P<0.0001$, Student's *t*-test). In both groups of mice, levels were undetectable at day 8. These data indicated that the serum half-life of hCFH was critically dependent on the degree of AP activation.

Administration of hCFH resulted in a rapid alteration in glomerular C3 staining in $Cfh^{-/-}$ mice

We next assessed renal C3 staining in $Cfh^{-/-}$ mice 24 h after a single intraperitoneal injection of 0.5 mg hCFH. $Cfh^{-/-}$ mice

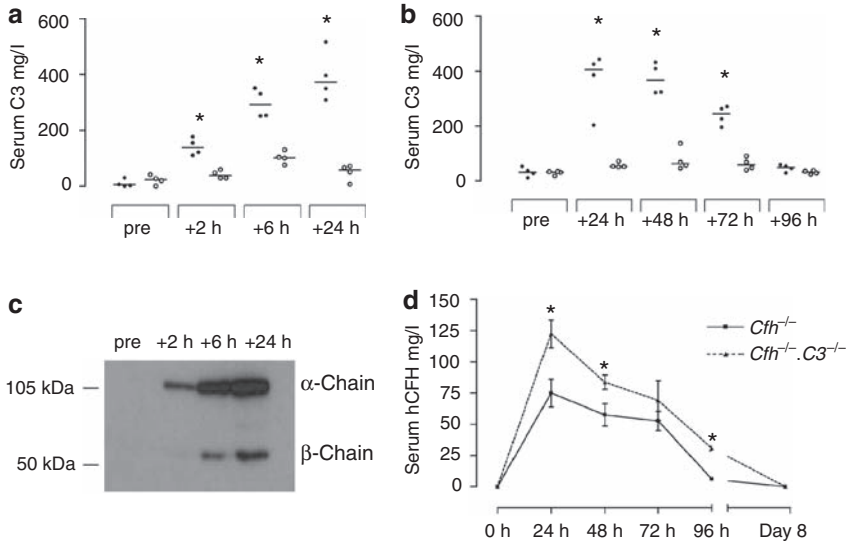

Figure 1 | Plasma C3 levels in $Cfh^{-/-}$ mice injected with hCFH. (**a, b**) Mouse serum C3 levels at different time points after a single intraperitoneal injection of either 0.5 mg hCFH (filled circles) or an equivalent volume of PBS (open circles). Panels **a** and **b** represent two separate experiments. Horizontal bars denote median values. Asterisk (*) denotes $P=0.0286$, Mann–Whitney test. In this ELISA pooled normal wild-type C3 level was 420 mg/l. (**c**) Representative western blot of sera under reducing conditions for C3 from a $Cfh^{-/-}$ animal injected with hCFH. (**d**) Serum hCFH levels after a single intraperitoneal injection of 0.5 mg hCFH into $Cfh^{-/-}$ ($n=4$, squares, solid line) and $Cfh^{-/-}.C3^{-/-}$ ($n=3$, triangles, dotted line) mice. Data points represent mean ± s.d. Asterisk (*) denotes $P<0.05$, Student's *t*-test. ELISA, enzyme-linked immunosorbent assay; hCFH, human complement factor H; PBS, phosphate-buffered saline.