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Abnormal Immune Complex Processing and Spontaneous Glomerulonephritis in Complement Factor H-Deficient Mice with Human Complement Receptor 1 on Erythrocytes

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Complement receptor 1 (CR1) on human erythrocytes (Es) and complement factor H (CFH) on rodent platelets perform immune adherence, which is a function that allows the processing of immune complexes (ICs) bearing C3 by the mononuclear phagocyte system. Similar immune adherence occurs in the glomerular podocyte by CR1 in humans and CFH in rodents. As a model for human IC processing, we studied transgenic mice lacking CFH systemically but with human CR1 on Es. These CR1huTg/CFH−/− mice spontaneously developed proliferative glomerulonephritis, which was accelerated in a chronic serum sickness model by active immunization with heterologous apoferritin. ICs containing Ag, IgG and C3 bound to Es in CR1huTg/CFH−/− mice. In this setting, there was increased IC deposition in glomeruli, attributable to the presence of CR1 on Es, together with the absence of CFH on platelets and podocytes. In the absence of plasma CFH, the accumulated ICs activated complement, which led to spontaneous and chronic serum sickness-induced proliferative glomerulonephritis. These findings illustrate the complexities of complement-dependent IC processing by blood cells and in the glomerulus, and the importance of CFH as a plasma complement regulator. The Journal of Immunology, 2010, 185: 000–000.

The C system contains over 30 plasma and cell-associated proteins, many of which are alike as a consequence of gene duplication events during evolution (1). Activation through classical, alternative or lectin C pathways leads to the cleavage of C3 and C5 and generation of C3a, C3b, C5a, and C5b-9. Complement is the first line of defense against some microorganisms and is an integral component of innate and adaptive immune responses to many others. Complement proteins are also important to clear immune complexes (ICs) and material derived from apoptotic cells; in doing so, they can shape the immune response to diverse Ags, including those derived from self and allogeneic tissue (1–3).

The regulators of complement activation proteins are composed of short-consensus repeat domains of ∼60 aa, and they are highly related within and between even distant species (4–6). The functional activities of these family members are attributable to their binding to C4 and C3 products (3, 7). Human complement receptor 1 (CR1)(CD35) is a type 1 transmembrane protein containing 30 short consensus repeats in its most common allele, which is present on erythrocytes (Es), neutrophils, monocytes, eosinophils, B cells, some T cells, follicular dendritic cells, and glomerular podocytes (8). The binding of C-opsonized particles to human Es, in a reaction termed immune adherence (9), is attributable to CR1. C3b-opsonized ICs bound to E CR1 are trafficked to cells of the mononuclear phagocyte system for removal from the circulation (10, 11). CR1 expression on Es is reduced in IC diseases, such as systemic lupus erythematosus, which may account for the tendency to deposit ICs in sites, such as the renal glomerulus (12, 13).

Complement factor H (CFH) is an important fluid phase C regulator (14, 15). CFH abnormalities can underlie human atypical hemolytic uremic syndrome, dense deposit disease (DDD), and age-related macular degeneration, with an ever-growing list of responsible mutations (3, 16). As a general rule, DDD is attributable to type I mutations leading to reduced functional plasma CFH and the ensuing unrestricted systemic alternative pathway activation, whereas atypical hemolytic uremic syndrome is attributable to type II mutations clustering in the terminal SCRs, leading to impaired ability of CFH to bind anionic sites, such as on endothelia, and to provide local protection against complement activation (3).

When CFH is absent in CFH−/− mice, animals develop a spontaneous inflammatory glomerular disease (i.e., glomerulonephritis [GN]), which leads to the late death of ∼25% of mice of mixed 129, C57BL/6, and DBA/2 backgrounds (17, 18). The genetic background clearly is important, because the 129 chromosome 1 has susceptibility loci (19). Histologically, glomeruli exhibit early C deposition, followed later by progressive IC deposits and glomerular hypercellularity, but without significant functional impairment of glomerular filtration or permeability to protein passage (17, 18). Consistent with alternative pathwaymediation, CFH−/− mice with a coexistent factor B deficiency are protected from disease (17). The spontaneous disease in CFH−/− mice requires C5 (but not C6) and C factor I, presumably to generate proinflammatory C5a and to create iC3b as ligand for β2-integrins on inflammatory cells, respectively (18, 20, 21). Whereas C57BL/6 CFH−/− mice have no evident glomerular disease, and wild type C57BL/6 CFH−/− mice are resistant to developing GN in chronic serum sickness.
(CSS) induced by repetitive immunization with heterologous apoferritin, all C57BL/6 CFH−/− mice develop GN in CSS (22).

Complicating the interpretation of studies in CFH−/− mice are our findings that CFH on platelets is responsible for immune adherence (23). Interestingly, a similar CFH for CR1 switch appears to occur in the mouse podocyte as well (24). Hence, in CSS, CFH−/− mice have greater deposition of ICs, yet in these circumstances, GN does not ensue unless CFH is absent in plasma, illustrating the importance of fluid-phase, and presumably local glomerular, C regulation in this model system (21).

To examine a system potentially more relevant to humans, in which E CR1 is responsible for IC processing, we used transgenic mice expressing human CR1 under a GATA1 promoter that drives expression in erythroid cells (CR1huTg) (25). We generated CR1huTg/CFH−/− mice to simulate and study IC processing by E-CR1. Surprisingly, although E-CR1 in these mice bound ICs, these ICs were trafficked to the kidney, leading to spontaneous development of severe GN in all mice, which was accelerated in CSS.

Materials and Methods

Animal experiments

CFH−/− mice [from Drs. Matthew Pickering and Marina Botto, Hammersmith Hospital, London, U.K. (17)] were crossed with CR1huTg mice bearing human CR1 on Es (25). Both mice were on the C57BL/6 genetic background (>N12). The resulting CR1huTg/CFH−/− mice lacked CFH on platelets and in plasma by FACS and Western blotting, respectively (22), whereas they had CR1hu on Es by FACS (25), which was not true of CFH−/− mice without the CR1huTg data shown).

The CSS model of IC-mediated GN was induced in CR1huTg/CFH−/− mice by actively immunizing mice with 4 mg horse spleen apoferritin (Sigma-Aldrich, Milwaukee, WI) given daily i.p. for 5 wk (22). Controls included CFH−/− and CR1huTg mice immunized with the same schedule of apoferritin and CR1huTg/CFH−/− mice receiving saline vehicle alone. At the conclusion of the 5-wk protocol, animals were euthanized for collection of serum, urine, and kidneys. In other experiments designed to identify apoferritin on Es and platelets by FACS and in kidneys by immunofluorescence (IF) microscopy, unbound apoferritin was replaced at different times with apoferritin labeled with Alexa 594 (Molecular Probes, Eugene, OR) to an average of five sites per molecule (26). For these experiments, animals were euthanized with blood and tissue collection 4 h after the final i.p. dose of apoferritin.

The spontaneous phenotype of CR1huTg/CFH−/− mice was also analyzed in a separate cohort of 17 mice, sacrificed at periodic intervals for collection of serum, urine, and kidneys. In all experiments, mice were housed in a specific pathogen-free facility. All studies with animals were approved by the University of Chicago Animal Care and Use Committee.

Flow cytometry

Platelets and Es were isolated and suspended in PBS with 10 mg/ml BSA (PBS/BSA) as described previously (23). Human CR1 was detected on Es using mAb 7G9 (IgG2a) labeled with Alexa 488 (Molecular Probes) following the manufacturer’s directions. Mouse IgG and C3 were detected with Alexa 488-labeled anti-mouse IgG and anti-mouse C3 Abs (Cappel, MP Biomedicals, Solon, OH). Individual Alexa 488–labeled Abs (100 μl of a 2 μg/ml stock) were added to 100 μl Es or platelets and incubated for 30 min at room temperature. Unbound Alexa 488-labeled Abs were removed by washing twice with PBS/BSA. Es and platelets were selectively gated by FACS based on forward- and side-scatter profiles; 20,000 events were counted using a FACSCalibur instrument (BD Biosciences, San Jose, CA) and analyzed with FloJo software (Tree Star, Ashland, OR).

Measurements from serum and urine

Blood urea nitrogen (BUN) concentrations were detected with a Beckman Autoanalyzer (Beckman Coulter, Fullerton, CA). Urinary albumin...
Concentrations were measured with a mouse albumin ELISA kit (Bethyl Laboratories, Montgomery, TX) and normalized to creatinine concentrations in the same urine (measured with Stanbio Laboratory Creatinine Procedure No. 0400; Boerne, TX).

Serum C3 was detected by Western blotting. Two microliters serum from individual animals was subjected to SDS-PAGE under reducing conditions, followed by electrophoretic transfer to a polyvinylidene fluoride membrane. C3 was detected with goat anti-mouse C3 IgG (Cappel) followed by IRDye 800 CW-labeled donkey anti-goat IgG (Rockland Immunochemicals, Gilbertsville, PA). Bound fluorescence was detected using an Odyssey Imager (LICOR Biosciences, Lincoln, NE). Of note is that with this approach, the anti-mouse C3 Ab appeared to react with the α-chain of mouse C3, but not the β-chain (Supplemental Fig. 1).

Anti-apoferritin IgG Abs were measured by ELISA as previously described (22). Polystyrene plates were coated with 5 μg/ml apoferritin. After blocking with 1% BSA, plates were incubated with serial dilutions of serum samples or anti-horse ferritin Ab (Jackson ImmunoResearch Laboratories, West Grove, PA), followed by HRP-conjugated anti-mouse IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and o-phenylenediamine peroxidase substrate (Sigma-Aldrich). The data were presented as arbitrary units relative to a standard curve generated with antihorse ferritin Abs.

To detect C3 in ICs, 5 μl serum from individual animals diluted in 95 μl PBS was added to a 20-μl slurry of protein G (Roche Applied Science, Indianapolis, IN). Samples were left overnight at 4°C on a rotator. Tubes were centrifuged at 2500 × g for 1 min at 4°C. Beads were washed three times with 500 μl ice-cold PBS. To minimize background the supernatant was completely removed after each wash. After the last wash, the pellet was vortexed and heated at 90–100°C for 5 min in 25 μl of 1× Laemmli reducing sample buffer. Proteins were separated by SDS-PAGE and electrophoretically transferred to a polyvinylidene fluoride membrane. Membranes were incubated sequentially with goat anti-mouse C3 (Cappel), IRDye 800 CW-labeled donkey anti-goat IgG, and Alexa 680-labeled goat anti-mouse IgG (Rockland, Gilbertsville, PA). Blots were washed with PBS/0.1% Tween-20, and fluorescence detected using an Odyssey Imager (LICOR Biosciences, Lincoln, NE).

Measurements from renal tissue

To evaluate pathologic renal changes, kidney tissues were fixed in 10% buffered formalin, embedded in paraffin, and 4-μm sections were stained with periodic acid-Schiff. The extent of GN was graded by an observer masked to the origin of the slides on a scale of 0 to 4 (in increments of 0.5) as described previously (22).

**FIGURE 2.** Features of humoral immune responses in CFH<sup>−/−</sup> and CR1<sub>hu</sub>Tg mice actively immunized with apoferritin. CFH<sup>−/−</sup>, CR1<sub>hu</sub>Tg, and CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice were actively immunized for 5 wk with apoferritin; as controls, CR1<sub>hu</sub>Tg mice received saline instead of apoferritin. A, Flow cytometry for platelet-associated (left panel) and E-associated (right panel) IgG. Data are representative of experiments performed three times. B, Quantification of serum antiaferritin IgG Abs by ELISA. Data from individual mice are shown. *p < 0.007 versus three other groups by ANOVA followed by Tukey’s pairwise comparisons. C, Presence of C3 in circulating immune complexes. Serum IgG was precipitated with protein G, followed by SDS-PAGE under reducing conditions and immunoblotting with anti-mouse C3 (green) and IgG (red) Abs. Each lane is from an individual mouse. Molecular weight standards are indicated on the left.
development of GN in every mouse. In contrast, wild type CFH-sufficient mice are uniformly resistant to GN in this model (22). To analyze how IC processing by E-associated CR1 might affect development of glomerular disease in this model, CSS was induced in a group of five CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice. As controls, CR1<sub>hu</sub>Tg (n = 4) and CFH<sup>−/−</sup> mice (n = 3) were also immunized with apoferritin at the same time, and as an additional control CR1<sub>hu</sub>Tg mice (n = 2) received vehicle alone.

As is known to be the case, there was a marked reduction in serum C3 levels in CFH<sup>−/−</sup> mice (17, 24). This acquired hypocomplementemia was not affected by the presence of E-associated CR1 in CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice, nor was it altered in the CSS model. CR1<sub>hu</sub>Tg mice had detectable C3, which did not appear to be affected by the CSS model (Supplemental Fig. 1).

All CFH<sup>−/−</sup> mice with CSS induced by active immunization with apoferritin for 5 wk developed GN characterized by proliferation within the mesangial and capillary regions and infiltration with inflammatory cells (Fig. 1B, 1D). Despite inflammatory GN, there was no impairment in glomerular filtration as determined by BUN measurements at the end of disease (Fig. 1A). These findings are consistent with our previous results in CFH<sup>−/−</sup> mice with CSS (22). Like wild type C57BL/6 mice, CR1<sub>hu</sub>Tg mice with intact CFH did not develop GN in this model (Fig. 1B, 1C). Surprisingly, CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice with CSS had functional renal impairment (BUN > 40 mg/dl in four of the five animals; Fig. 1A) associated with severe proliferative GN (Fig. 1E, 1F). All five animals in this study had GN scores ≥ 3.0 (Fig. 1B), indicating both endocapillary and extracapillary (i.e., crescentic) GN (Fig. 1F).

**IC processing in CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice with CSS**

To analyze relevant features of the immune response in CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice, platelet- and E-associated IgG and serum antiapoferritin IgG Abs were measured in animals from the above studies. In these and subsequent experiments described below, studies were performed 4 h after the final i.p. dose of apoferritin, which presumably was absorbed from the peritoneum over this time. As shown in Fig. 2A, apoferritin-immunized CR1<sub>hu</sub>Tg but not CFH<sup>−/−</sup> mice had platelet-associated IgG, which is consistent with the importance of platelet-associated CFH to bind ICs. Interestingly, CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice immunized with apoferritin had some platelet-associated IgG, perhaps owing to platelet-associated CR1<sub>hu</sub> (25). Only CR1<sub>hu</sub>Tg mice immunized with apoferritin had E-associated IgG, with slightly less present in CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice relative to CR1<sub>hu</sub>Tg mice with intact CFH, possibly reflecting the acquired hypocomplementemia in the former, which could reduce IC binding to CR1. By this approach, there was no detectable IgG on platelets or Es of CR1<sub>hu</sub>Tg mice receiving vehicle as control.

All mice immunized with apoferritin, but not CR1<sub>hu</sub>Tg controls receiving vehicle alone, had antiapoferritin IgG Abs in sera (Fig. 2B). CFH<sup>−/−</sup> mice had significantly higher levels of antiapoferritin IgG compared with CR1<sub>hu</sub>Tg mice. Interestingly, CR1<sub>hu</sub>Tg mice had comparable antiapoferritin IgG levels irrespective of CFH status. Thus, the absence of CFH on platelets and the hypocomplementemia in CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice did not translate into greater circulating antiapoferritin IgG Abs relative to CR1<sub>hu</sub>Tg mice. In these analyses, it was not possible to exclude the capture of antiapoferritin IgG in ICs. That the higher apparent antiapoferritin IgG in CFH<sup>−/−</sup> mice could reflect the lack of platelet-associated CFH (and E-associated CR1) to bind antiapoferritin IgG in ICs (containing C3b) was examined in further studies. IgG in CFH<sup>−/−</sup> mouse serum contained C3b (presumably as the C3b β<sub>1b</sub> chain), whereas there was no C3 associated with IgG in CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice (Fig. 2C). These data support the

**Results**

**Glomerular disease features in CR1<sub>hu</sub>Tg/CFH<sup>−/−</sup> mice with CSS**

CSS induced by continued administration of heterologous apoferritin leads to IC accumulation in glomeruli in CFH<sup>−/−</sup> mice, with
finding that E-associated CR1 was capable of binding C3b-containing ICs.

To further analyze IC handling by CR1huTg/CFH−/− mice, variations of the CSS protocol were used in separate cohorts of CR1huTg/CFH−/− and CFH−/− mice. In initial experiments, 8-wk-old mice (n = 3 per group) were immunized with unlabeled apoferritin, as before for 3 wk. For the final 2 wk of the protocol, mice were immunized with Alexa 594-labeled apoferritin. Glomerular deposition of the labeled Ag was then evaluated together with detectable apoferritin (Fig. 4A) from both CR1huTg mice and CR1huTg/CFH−/− mice. Es from both groups, but not CFH−/− mice, had detectable C3 on the E surface (Fig. 4D). Consistent with these data with CR1huTg/CFH−/− mice, there was evidence for a small population of platelets bearing relatively large quantities of apoferritin. Es from both CR1huTg groups, but not CFH−/− mice, had detectable apoferritin (Fig. 4B). In another set of experiments, 8-wk-old CR1huTg/CFH−/−, CFH−/−, and CR1huTg mice (n = 3 per group) were immunized with unlabeled apoferritin for 3 wk and then given a final immunization with Alexa 594-labeled apoferritin. Four hours later, animals were sacrificed for analyses of platelet-, E-, and glomerular-associated apoferritin and C3. As shown in Fig. 2A, Alexa 594-apoferritin was present on platelets from CR1huTg but not CFH−/− mice. In CR1huTg/CFH−/− mice, there was evidence for a small population of platelets bearing relatively large quantities of apoferritin. Es from both CR1huTg groups, but not CFH−/− mice, had detectable C3 on the E surface (Fig. 4D). Surprisingly, C3 was not evident on platelets from any of the groups, including those with intact CFH (i.e., CR1huTg; Fig. 4C). These data support the conclusion that human CR1 on CR1huTg mouse Es bound ICs containing apoferritin and mouse C3, even in the relatively low-circulating C3 environment present in CR1huTg/CFH−/− mice.

In glomeruli of CR1huTg mice, there was only a slight accumulation of Alexa 594-apoferritin with the mesangial regions, together with C3 and IgG (Fig. 5A). As expected, CFH−/− mice had extensive deposits of C3 in the capillary wall, whereas Alexa 594-apoferritin and IgG were centrally present within mesangial regions of glomeruli (Fig. 5C). That the glomerular C3 was relatively independent of the abbreviated CSS protocol was shown by experiments with CFH−/− mice receiving saline instead of apoferritin over 3 wk followed by Alexa 594-apoferritin as in the other groups (Fig. 5B). Finally, in CR1huTg/CFH−/− mice, there was extensive deposition of Alexa 594-apoferritin and IgG within glomeruli, extending to the peripheral capillary walls (Fig. 5D, 5E, arrows). Thus, these data expand on the findings that CR1huTg/CFH−/− mice have abnormal IC metabolism, leading to short-term and long-term IC deposition in glomeruli.

**Spontaneous development of GN in CR1huTg/CFH−/−**

Given the abnormal IC processing and development of severe GN in CR1huTg/CFH−/− mice with CSS, we studied a group of unmanipulated 17 CR1huTg/CFH−/− mice. Over time, these animals developed progressive renal functional impairment, characterized by albuminuria (Fig. 6A) and azotemia, as documented by elevated BUN values (Fig. 6B). Of the mice analyzed for over 20 wk, two of five died (25 and 28 wk), and the remaining three had significant renal failure at 30 wk, with BUN values of 44, 60, and 90 mg/dl. Hematocrits remained ≥39 except for one 30-wk-old animal with advanced renal failure (BUN = 90, in which the hematocrit was 26).

Histopathologically, CR1huTg/CFH−/− mice developed diffuse endocapillary proliferative GN which became progressively more severe over time from 8 to 30 wk. At the later ages, in addition to endocapillary proliferation, there was extracapillary proliferative GN (GN scores ≥3.0). Fig. 7A–F show representative glomerular histopathology in the different age groups, and Fig. 7G shows GN scores for all animals. Only glomeruli were involved, except in the

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**FIGURE 4.** ICs on CFH−/− and CR1huTg mouse platelets and Es in CSS. CR1huTg, CFH−/− and CR1huTg/CFH−/− mice (n = 3 each) were actively immunized for 3 wk with unlabeled apoferritin, followed by a final dose of Alexa 594-labeled apoferritin. Four hours later, pooled platelets (A, C) and Es (B, D) from each group were analyzed by FACS for Alexa 594-labeled apoferritin (A, B) and mouse C3 (C, D).
30-wk-old mouse with renal failure, which also had tubulointerstitial nephritis and arteritis.

Immunoreactants in glomeruli were also examined by IF microscopy. As is typical for \( CFH^{-/-} \) mice (17), there was considerable C3 present in a linear staining pattern in peripheral capillary walls of all \( CR1_{huTg}/CFH^{-/-} \) mice (Fig. 8A–F). However, there was also accumulation of IgG; initially, this was in mesangial regions and appeared to be in locations distinct from the C3 staining (Fig. 8C, 8D, arrows). At later ages, IgG extended to peripheral capillary walls, including in thickened areas of the glomerular capillary wall (Fig. 8E, 8F, arrowheads), which is often associated with immunoreactive C3. There was minor mesangial accumulation of IgM only at the later ages, and little to no staining for IgA in glomeruli in all animals (Fig. 8G). Thus, \( CR1_{huTg}/CFH^{-/-} \) mice had abnormal IC processing independent of active immunization with exogenous Ag, leading to the spontaneous development of GN.

Discussion

In humans, CR1 on Es is the immune adherence receptor, responsible for binding C3-containing ICs and properly transporting them to the mononuclear phagocyte system (8–11). In contrast, rodents rely on platelets for this function, which can be attributed to CFH (23). Thus, we hypothesized that creating mice with CR1 on Es, but lacking CFH on platelets, would be physiologic and allow more direct comparisons to humans. To our surprise, \( CR1_{huTg}/CFH^{-/-} \) mice developed proliferative GN, which was fatal in some, and could be accelerated in the CSS model. This finding contrasts with the spontaneous development of a form of GN (i.e., DDD) in a minority of older \( CFH^{-/-} \) mice in a strain-dependent fashion (17). Thus, the presence of human CR1 on Es of \( CFH^{-/-} \) mice proved to be detrimental, with development of progressive GN in these animals. This development was severe enough to lead to the death of animals over 25 wk of age. Thus, rather than being physiologic, the presence of human CR1 on Es promoted pathologic events in \( CFH^{-/-} \) mice.

Regardless of the presence or absence of CFH, \( CR1_{huTg} \) mice immunized with apoferritin had IgG, C3, and Ag associated with Es. It seems likely these were present in ICs containing mouse C3b bound to human CR1. Consistent with a role for platelet-associated CFH as the immune adherence receptor, mice with intact CFH also had IgG and apoferritin Ag on platelets in the CSS model. Despite the relatively large amounts of IgG and Ag on these platelets, and that C3 could be detected on Es of \( CR1_{huTg} \) mice, C3 was not detectable on platelets in these mice, for which we do not have a clear explanation. In the CSS model, circulating antiapoferritin IgG Abs were greater in mice lacking both systemic CFH and E CR1, whereas they were equivalent in mice with E CR1 regardless of whether CFH was present.

**Figure 5.** ICs in \( CFH^{-/-} \) and \( CR1_{huTg} \) mouse glomeruli in CSS. \( CR1_{huTg} \) (A), \( CFH^{-/-} \) (C), and \( CR1_{huTg}/CFH^{-/-} \) mice (D, E, \( n = 3 \) each) were actively immunized for 3 wk with unlabeled apoferritin, followed by a final dose of Alexa 594-labeled apoferritin. As control, \( CFH^{-/-} \) mice receiving saline instead of apoferritin over the 3 wk period also received Alexa 594-labeled apoferritin (B). Four hours later, kidneys from each group were analyzed by IF for Alexa 594-apoferritin (red), mouse IgG (blue), and mouse C3 (green). The merged images of representative glomerular IF staining are shown. Arrows indicate Alexa 594-apoferritin and IgG in glomerular peripheral capillary walls (original magnification \( \times 600 \)).

**Figure 6.** Development of biochemical features of renal disease over time in \( CR1_{huTg}/CFH^{-/-} \) mice. Shown is albuminuria (A) and BUN (B) in \( CR1_{huTg}/CFH^{-/-} \) mice over time. Each symbol represents data from an individual mouse.
present. Moreover, free IgG-C3b complexes were present only in the sera of CFH$^{-/-}$ mice. These data further support a role for E CR1 in antiapoferritin IgG handling in the CSS model.

CR1huTg/CFH$^{-/-}$ mice had excessive IgG-containing ICs in glomeruli spontaneously and in the CSS model. In the former instance, the Ag could not be characterized, whereas in the latter it clearly contained apoferritin. This was not simply a consequence of the Ag position occurring in mice with platelet CFH resulted in GN in plasma CFH was absent (21). Moreover, even limited IC deposition occurring in mice with platelet CFH resulted in GN in animals in which plasma CFH was absent.

Our short-term studies following labeled apoferritin Ag in the CSS model provided further evidence for abnormal IC handling by mice lacking CFH (i.e., CFH$^{-/-}$ and CR1huTg/CFH$^{-/-}$ mice). In CR1huTg mice, ICs were present on both platelets and Es. In CR1huTg/CFH$^{-/-}$ mice, they were present primarily on Es, and in CFH$^{-/-}$ mice, they were absent from both platelets and Es. CR1huTg mice had only mild deposition of ICs within the glomerular mesangium, whereas CFH$^{-/-}$ mice had quantitatively greater IC deposits. Overall, findings these are consistent with our previous data showing that platelet-associated CFH is necessary for IC processing (21). Yet, in CR1huTg/CFH$^{-/-}$ mice, there was substantially greater glomerular IC deposition with extension to the peripheral capillary wall. This deposition occurred despite IC binding to Es in these mice. Thus, only in the situation in which CR1 was present on Es and CFH be absent systemically.

As occurs in CFH$^{-/-}$ mice (17), young CR1huTg/CFH$^{-/-}$ mice had substantial C3 in the glomerular capillary wall. Early in the spontaneously occurring disease in CR1huTg/CFH$^{-/-}$ mice, ICs tended to be present in mesangial regions that did not have C3. With time, ICs progressively accumulated in the peripheral capillary wall, which initially may have replaced C3 in these areas. Yet, in both spontaneous GN in older CR1huTg/CFH$^{-/-}$ mice and in the CSS model, glomerular ICs also contained adjacent C3 staining. Overall, we would argue the development of GN in CR1huTg/CFH$^{-/-}$ mice was attributable to increased ICs in glomeruli, which activated C in an unrestricted C fashion because CFH was absent from plasma. Our previous studies with CSS in bone marrow chimeric CFH$^{-/-}$ mice support the conclusion that excessive IC deposition in glomeruli alone does not lead to inflammation or GN. In these studies, excessive glomerular IC deposition occurred because of absent platelet CFH; however, GN did not result because plasma CFH was present (21). Moreover, even limited IC deposition occurring in mice with platelet CFH resulted in GN in animals in which plasma CFH was absent.

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Likely because mouse C3 has regions conserved with human C3, human CR1 effectively binds mouse C3b in rosetting assays (27) and can bind mouse iC3 in a fluid-phase system, albeit ∼5-fold less than it does human iC3 (28). Thus, in the CSS model, CR1huTg mouse Es had surface C3, presumably as C3b in ICs. Because of unrestricted alternative pathway C activation, CFH$^{-/-}$ mice have low plasma C3 levels (17, 24), which was also true of CR1huTg/CFH$^{-/-}$ mice. Yet,
C3 appeared to be activated on ICs in CR1huTg/CFH−/− mice, presumably reflecting the balance of low C3 available for classical pathway activation, yet enhanced amplification through the alternative C pathway, as also appears to occur in glomeruli in mice lacking plasma CFH (21). Related to this is the finding of lower quantities of E-associated C3 in CR1huTg/CFH−/− mice compared with CR1huTg mice. These data support the conclusion that CR1 on mouse Es in CR1huTg/CFH−/− mice was capable of binding mouse C3b-containing ICs from the circulation.

Beyond their ability to bind C3b, CFH and CR1 are both decay accelerators and cofactors for factor I-mediated inactivation of C3-bearing alternative pathway convertases. Human CR1 can be an effective complement inhibitor in mice in vitro and in vivo (29). Whether this is due to decay-accelerating and/or cofactor effects is not clear. It has also been shown that mouse factor I is compatible with human CR1 on Es for the cleavage of human C4b (30). Thus, the existing data provide strong evidence that human CR1 can bind mouse C3b, which is further supported by our present studies, whereas the explanation of whether human CR1 on mouse Es bearing C3b-containing ICs can recruit mouse factor I to lead to cleavage of C3b is not clear. C3 being present on CR1-transgenic mouse Es, but not CFH-bearing platelets, suggests that the functions of CR1 and CFH are dissimilar in these two sites. Beyond their species compatibilities, CFH does not effectively bind C4b (as in ICs) or serve as factor I cofactor for cleavage of iC3b to C3d. Thus, overall there are a number of reasons why human CR1 on mouse Es is not physiologically relevant to mouse CR1 on mouse Es alone could not account for the excessive glomerular deposition of ICs (and development of GN) spontaneously and in CSS in CR1huTg/CFH−/− mice. These findings raise interesting aspects for further study in this model system.

Although Es in CR1huTg/CFH−/− mice bound ICs, these animals had excessive glomerular ICs relative to CR1huTg and CFH−/− mice. The lack of plasma CFH did not appear to substantially affect IC binding to Es in CR1huTg/CFH−/− mice. Moreover, the presence of human CR1 on mouse Es alone could not account for the excessive glomerular ICs. Whereas the lack of CFH on platelets appears to contribute to glomerular IC deposition (21), there was a marked difference in glomerular ICs in CR1huTg/CFH−/− mice compared with CFH−/− mice. Thus, the lack of plasma and platelet CFH and the presence of human CR1 in CR1huTg/CFH−/− mice were not complete explanations for our findings of excessive glomerular ICs.

Besides Es, human glomerular podocytes use CR1 as an immune adherence receptor (31, 32) for reasons that have largely been obscure (33). As with Es, rodent podocytes appear to replace CR1 with CFH (34). Our previous studies using a renal transplantation approach in CFH−/− mice have shown that podocyte CFH facilitates the transcapillary passage of ICs in glomeruli (24). Interestingly, the neonatal FcR is also present on human and mouse podocytes, where it clears IgG from the glomerular capillary wall (35). An attractive explanation for our findings is that glomerular-deposited ICs are not cleared appropriately in the absence of podocyte CFH.

In summary, our studies concentrated on CR1huTg/CFH−/− mice, which had human CR1 on Es, yet lacked plasma, platelet, and podocyte CFH. These mice developed spontaneous proliferative GN, which could be accelerated in the CSS model. These disease features were unique relative to all other nonautoimmune strains studied in the past (17, 18, 20–22, 24, 36, 37). It appeared that the presence of human CR1 on Es altered IC processing in the CFH−/− mouse, such that there was increased IC deposition in glomeruli. Whether this finding is physiologic or pathologic is not clear based on our data. Nonetheless, these ICs were not processed through the glomerular capillary wall, wholly or in part because of the absence of CFH on podocytes. The accumulated ICs were proinflammatory in the absence of circulating CFH, which led to spontaneous and CSS-induced severe proliferative GN.

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Disclosures

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References


