Isolation and Characterization of a Novel Rat Factor H-related Protein That Is Up-regulated in Glomeruli under Complement Attack*

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The factor H family in humans is composed of seven distinct proteins, including factor H-related proteins (FHR) 1–5. All members contain tandemly arranged short consensus repeats (SCR) typical of the regulators of complement activation gene family. FHR-5 is unusual for this group of proteins, as it was initially identified as a component of immune deposits in glomerular diseases. During our cloning of the cDNA for rat factor H from glomerular epithelial cells (GEC), we identified an alternative 2729-bp cDNA transcript. The translated sequence encoded a protein containing 11 SCRs, most similar to SCRs 7–15 and 19–20 in native rat factor H, which is the same basic structure of human FHR-5. As such, this rat protein was termed FHR. Recombinant rat FHR produced in a eukaryotic expression system had a molecular mass of 78 kDa. In functional studies, recombinant FHR bound C3b and inhibited the complement alternative pathway in a dose-dependent fashion. Given the prominent expression of FHR-5 in human membranous nephropathy, a disease in which complement activation occurs in the vicinity of GEC, the expression of FHR in a rat model of this disease was evaluated. In both in vitro and in vivo models of complement activation on the GEC, FHR mRNA was up-regulated by a factor of 3–6-fold compared with controls in which complement activation could not be activated. Thus, we have identified a novel factor H family member in rats. This FHR protein is analogous to human FHR-5, both in structure and in potential involvement in glomerular immune complex diseases.

Activation of the complement cascade leads to the production of a number of proteins that contribute to inflammation. This is beneficial in host defense but can be detrimental if activated on self tissue. To prevent this, a number of naturally occurring complement regulatory proteins are present to restrict complement activation throughout the cascades of the three pathways (1). A focal point of regulation is at the level of C3/C5 convertases. This occurs in humans via the actions of the plasma proteins, factor H- and C4-binding protein, and the cell membrane proteins, complement receptor 1, decay-accelerating factor, and membrane cofactor protein, all members of the regulators of complement activation gene family located on chromosome 1q32 (2). All family members have common 60–70-amino acid short consensus repeats (SCR), containing four invariant cysteines that form two intra-SCR disulfide bonds (2). These proteins have natural affinity for C3b and/or C4b which confers upon them the ability to accelerate the intrinsic decay of C3/C5 convertases and/or act as a necessary cofactor for the cleavage and inactivation of C3b and C4b by factor I. Human factor H was originally isolated in 1965 as $\beta_1$H-globulin by Nilsson and Müller-Eberhard (3), and 10 years later its function was determined by both the Ruddy and Fearon laboratories (4–6). Over the ensuing 20 years, five proteins similar to factor H have been identified, including factor H-like protein 1 and factor H-related proteins (FHR) 1–4, thus making this a family of closely related proteins (7). These proteins are synthesized primarily in the liver and are secreted into plasma (7). Factor H, FHR-5, and FHR-4 have been shown directly to have affinity for C3b (8–10). Of these proteins, the function of factor H has been best characterized. It inhibits the formation and accelerates the decay of C3 convertases, serves as a cofactor for factor I (11), displays chemotactic activity for monocytes (12), can associate with extracellular matrix and leukocytes (13), and interacts with a variety of ligands, such as heparin, C-reactive protein, and adrenomedullin (14, 15).

Activation of the complement cascade appears to contribute to immunologically mediated glomerular diseases (16). Circumstantial evidence for this is the presence of complement activation products in glomeruli and in urine (17–19). The use of animal models for glomerular diseases has strengthened the case for a role of complement under these conditions. Among the most widely used model of any glomerular disease is the passive Heymann nephritis (PHN) rat model of human membranous nephropathy. PHN is induced by injection of heterologous anti-Fx1A antibodies that progressively accumulate on the glomerular epithelial cell (GEC) (20). Once a threshold amount of anti-Fx1A has accumulated, complement-dependent damage to the GEC occurs leading to impairment of the glomerular permselectivity barrier to protein passage with consequent development of proteinuria (21–23). Murphy and co-workers produced a number of monoclonal antibodies against nephritic glomeruli (24), which allowed them to clone the cDNA for clusterin (25). More recently, an...

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1 The abbreviations used are: SCR, short consensus repeat; BCECF, bis(carboxyethyl)carboxyfluorescein; CVF, cobra venom factor; FHR, factor H related protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GEC, glomerular epithelial cells; MTT, methythiazole tetrazolium; PHN, passive Heymann nephritis; RACE, rapid amplification of cDNA ends; BSA, bovine serum albumin; PBS, phosphate-buffered saline.
other one of these monoclonal antibodies was used to clone the fifth FHR (FHR-5) (26). Although FHR-5 was not expressed in normal glomeruli, it was uniformly expressed in the glomeruli of patients with immunologically mediated diseases, such as membranous nephropathy (27). Whether FHR-5 appeared in glomeruli from plasma and/or was locally produced was not determined in these studies.

In our attempt to clone factor H from rat GEC, we identified its functional properties and its expression in normal and diseased glomeruli and GEC.

**EXPERIMENTAL PROCEDURES**

**Cloning of a Partial Length cDNA for Rat FHR**—In initial experiments, PCR primers, GSP-F1 and GSP-R1 (Table I), were designed from the known sequence of mouse factor H (GenBank accession number M12690), spanning bases 2556–3860, which corresponds to SCRs 14–20 of mouse factor H. Total RNA was isolated from cultured rat GEC using TRIzol reagent (Invitrogen). cDNA was produced from 5 μg of total RNA by reverse transcription using oligo(dT) primers (Invitrogen). Throughout the cloning, cDNA from rat liver and glomeruli (isolated as described below) were processed identically as PCR controls. The PCR product was performed with 20 mM Tris-HCl, 2 mM MgCl2, 100 μM of each deoxynucleotide triphosphate, 0.2 μM of each primer, and 2.5 units of Taq polymerase. Thirty five cycles of 1 min denaturation at 94 °C, 1 min annealing at 55 °C, and 1 min extension at 72 °C were performed. PCR products generated were electrophoresed through a 1.5% agarose gel and stained with ethidium bromide. Fusion PCR products were cloned using LipofectAMINE Plus Reagent (Invitrogen) and the abridged universal amplification primer. The sequence provided the putative ATG initiation signal. The 3′- and 5′-RACE products were combined to generate the full-length cDNA.

**Expression of Recombinant Rat FHR**—The open reading frame for FHR was amplified from cDNA by E-LONGASE Enzyme Mix using primers PCD-F and PCD-R in Table I. The PCR product was cloned into a PCD vector, pDONR221 (Invitrogen). The resulting plasmid (pDONR221-FHR) was transformed into competent E. coli (One Shot TOP10, Invitrogen). Positive clones were selected on LB agar containing 50 μg/ml for selection of transfected cells. Clonal CHO-K1 cell lines stably expressing FHR were expanded in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 500 μg/ml G418. Because of the potential confounding effect of factor H and other family members in serum, the cells were then adapted to serum-free conditions using ProCHO-CDM serum-free cell culture medium (BioWhittaker, Walkersville, MD). Batch suspension culture was established in glass spinner flasks with an inoculum of 2 × 106 cells/ml. Cell-free supernatants were concentrated using Centricron Plus-30 concentrators (Millipore, Bedford, MA), and the His6-tagged recombinant FHR was then purified by chromatography on HisTrap and HiTrap desalting columns (Amersham Biosciences) according to the manufacturer’s protocol. The eluted FHR was concentrated in a Centricron YM-30 concentrator (Millipore). For affinity purification of anti-FHR antibodies, 0.5 mg of FHR was bound to 1 ml of CNBr-Sepharose (Amersham Biosciences) utilizing the standard protocol recommended by the manufacturer.

**Western Blotting**—Affinity-purified proteins or normal rat serum (diluted 1:800 in PBS) were separated by SDS-PAGE and electrophoretically transferred overnight to a polyvinylidene difluoride membrane (Millipore). The membrane was blocked with 5% (w/v) nonfat dry milk in 0.1 M NaCl, 0.02 M Tris, pH 7.2, 0.5% Tween 20 (TBST) for 2 h. The membrane was incubated overnight with a monoclonal antibody to C-terminal polyhistidine (anti-His(C-term), Invitrogen) (1:2000) or goat polyclonal antisera to human factor H (Quidel Corp., San Diego, CA) (1:3000), which is cross-reactive with rat factor H (28). In one set of experiments, this anti-human factor H antisera was passed over a column of rat FHR-Sepharose, and the eluted affinity-purified anti-rat FHR antibodies were used. The antibodies were washed three times with TBST, incubated with peroxidase-conjugated anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) (1:30000), or peroxidase-conjugated anti-goat IgG (Sigma), followed by washing with TBST. Bound antibody was detected with SuperSignal Fico chemiluminescent substrate (Pierce).

In Vitro Binding Studies with Recombinant Rat FHR—Recombinant rat FHR or BSA was serially diluted in 0.1 M carbonate buffer, pH 9.3. Duplicate wells in microtiter plates were incubated with dilutions of FHR from 400 to 3.1 ng/well. The plates were blocked with BSA in PBS, and wells were incubated with rat C3b (10 μg/well) (29). C3b was detected by incubation with polyclonal goat anti-rat C3 antibody (Cap- sas) (Cappel, OR) (1:10000 dilution) followed by peroxidase-conjugated rabbit anti-goat IgG antibody (Sigma) (1:2000 dilution). Peroxidase activity toward added o-phenylenediamine dihydrochloride (Sigma) was detected at a wavelength of 450 nm.

**Complement Alternative Pathway Inhibition**—The capacity of recombinant FHR to inhibit alternative pathway activation was studied. In initial experiments, factor H and FHR were bound to the polystyrene surface from rat serum by passage over a column of anti-factor H bound to CNBr-Sepharose. Added to a total reaction volume of 0.5 ml of DGVB-MgEGTA (72.5 mM NaCl, 2.5 mM sodium barbital, 4 mM MgCl2, 10 mM EGTA, 2.5% dextrose, 0.1% gelatin, pH 7.4) was 10 μl of this serum, 1 × 107 guinea pig erythrocytes, and serial dilutions of rat FHR. After 90 min at 37 °C, the reaction was stopped by the addition of 10 mM EDTA containing 10 mM EDTA; erythrocytes were pelleted, and the degree of hemolysis was determined by measuring the A412 of the supernatant. The percent inhibition of hemolysis by FHR was compared with reactions in which no FHR was added.
Northern Analyses—20 μg of total RNA from rat liver was electrophoresed through a 1% agarose gel containing 2.2 M formaldehyde, transferred by capillary action to a nylon membrane, and cross-linked by ultraviolet irradiation. Membranes were prebhibited and hybridized at 42 °C in buffer containing 5 × SSPE, 5 × Denhardt’s solution, 200 μg/ml of denatured salmon sperm DNA, 0.1% SDS, and 50% formamide. Specific probes were random primer-labeled with [α-32P]dCTP to activities of ~107 cpm/μg (Roche Molecular Biochemicals). After hybridization with the probes, the membranes were washed with 2 × SSC, 0.1% SDS and then at 65 °C with 0.1 × SSC, 0.1% SDS. Autoradiography was subsequently performed at ~80 °C with an intensifying screen.

FHR Expression in Vivo—The expression of FHR mRNA was evaluated in cultured rat GEC in which complement was activated. To make these as relevant to PHN as possible, anti-Fx1A was used as the complement-activating antibody, and conditions were chosen to lead to cell injury but not death (so-called sublytic injury) (30). To measure cell injury, cells were loaded with 5 μM bisacarbonyl carboxyfluorescein (BCECF) as its membrane-permeant acetoxymethyl ester form (Molecular Probes, Eugene, OR). Once intracellular, this is deesterified to BCECF which is retained by normal cells. Disruption of plasma membrane integrity by C5b-9 leads to release of ~520-dalton marker into the cell medium which is quantified spectrofluorimetrically (30). To measure cell death, the methylthiazole tetrazolium (MTT) colorimetric assay was utilized according to the manufacturer’s instructions (Sigma). Dose-response studies indicated that 2.5 mg/ml anti-Fx1A and 2% (v/v) normal human serum were optimal. Even in high concentrations, rat serum did not lead to appreciable cell injury, which can be attributed to intrinsic complement regulators that restrict homologous complement activation (31, 32).

Confluent GEC in 12-well culture dishes were exposed to 2.5 mg/ml anti-Fx1A in PBS for 30 min at 22 °C. After washing, cells were exposed to 2% human serum at 37 °C. RNA was isolated 1, 2, and 4 h following exposure to serum (in triplicates for each time point). A group of control cells was treated identically yet exposed to serum that had been heated previously at 56 °C for 30 min to inactivate complement. For all variables, parallel sets of cells were treated identically, except that they were loaded with BCECF at the same time as antibody sensitization, supernatants were collected for BCECF measurements, and cells were used for MTT uptake.

**FHR Expression in Vivo**—The PHN model of membranous nephropathy was used to evaluate the effects of complement activation on FHR expression in glomeruli. PHN was induced by standard techniques in normal rats by injection of anti-Fx1A at a nephritogenic dose (33). As a control, animals were depleted of complement by a series of injections of cobra venom factor (CVF) (34). Animals were given 90 units of CVF (Quidel, Santa Clara, CA) divided into three equal intraperitoneal doses on the day prior to antibody administration and then units daily for the duration of the study. Adequacy of complement depletion was determined by measuring the total hemolytic activity of serum after CVF treatment by standard techniques (35) and comparing it to pretreatment values. The complement-replete animals were given buffer injections instead of cobra venom factor. At 3–6 days following injection of anti-Fx1A, glomeruli were isolated by a technique in which iron oxide particles were injected into the renal artery (36) followed by magnetic purification, which resulted in a purity of >99% by phase contrast microscopy. For every time point, 3 rats were studied.

**Real Time Quantitative PCR**—Quantitative PCR was done on reverse-transcribed cDNA from all samples. The primers and probe for FHR were 5′-GAGTTCTCAGATGGAGGAGG-3′ and 5′-GCCACATT-TCCCTGTTGATT-3′. 5′-FAM-AATTTGGGCTCTCGGCTGTT-TAMRA-3′ (Synthegen, Houston, TX). As a commonly used housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize RNA from each sample, using a primer and probe set from Applied Biosystems (Foster City, CA). Quantitative PCR was done by standard techniques using a Cepheid Thermocycler. Standard curves were constructed for cultured rat GEC and isolated glomeruli. The target messages in unknown samples were quantified from the standard curves to determine a relative measure of the starting transcript quantity. Each sample was then normalized to its own GAPDH expression, for which a similar standard curve had been constructed. All samples were calculated relative to the control sample that was designated as 1.0.

**Statistical Methods**—Data are shown as mean ± S.E. Comparisons were made with analysis of variance followed by Fisher’s pairwise comparisons utilizing Minitab software (College Park, MD).

**RESULTS**

**Cloning of Rat FHR cDNA**—Our initial efforts were directed toward cloning rat factor H, which we had identified in liver, platelets (28), and cultured GEC (not shown). The initial PCR strategy was to design primers from regions in SCRs 14 and 20 of mouse factor H, with the rationale these areas would be conserved in the rat. When cDNA prepared from GEC, normal rat glomeruli, and liver RNA was amplified with the primers, two products with 1057 and 529 bp were generated (Fig. 1). The nucleotide sequence of the 1057-bp product was identical to the newly deposited sequence for rat liver factor H (GenBank™ accession number AJ320522), whereas the 529-bp product encoded a protein with 80–90% homology to SCRs 14, 15, 19, and 20 from rat factor H. With this single sequence, the subsequent identification of the full-length cDNA clone was achieved using the RACE strategy outlined under “Experimental Procedures.” By 5′- and 5′-RACE, 984- and 1523-bp products were obtained, respectively. Sequencing of these clones enabled us to generate the full-length cDNA. Fig. 2 shows the position and overlap of these clones relative to the 2729-bp full-length cDNA.

The complete cDNA sequence and deduced amino acid sequence are shown in Fig. 3 (GenBank™ accession number AF436847). There was an open reading frame from bases 50 to 90% homology to SCRs 14 and 20, a mature protein of 676 amino acids. This was followed by 568 bases of 3′-untranslated sequence, including a consensus polyadenylation signal at positions 2610–2615 and a poly(A) tail. As this protein is related to factor H, and has features in common with the human FHR family members, it was termed rat FHR.

Mature rat FHR has a predicted nonglycosylated molecular mass of 75,715 daltons and two potential N-linked glycosylation sites at positions 136 and 388 (Fig. 3). There are 11 SCRs, each containing the four characteristic cysteine residues and additional conserved amino acids. A comparison between rat factor H and FHR is shown in Fig. 4. Also apparent is that nine SCRs in rat FHR have 24–79% homology to the nine SCRs in the human FHR-5 protein.

**Northern Blot Analysis**—To confirm the presence and size of rat FHR RNA and compare it to that from factor H, liver RNA was probed with specific nucleotide products obtained during the cloning of FHR and factor H. As shown in Fig. 5, the 984-bp 3′-RACE product (as schematically depicted in Fig. 2) hybridized with a single mRNA species with an estimated size of 2.7 kb (lane 1). By way of comparison, a specific 3′ probe for factor

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**Fig. 1.** PCR-based approach to amplify factor H and FHR cDNA. Two distinct PCR products were generated from GEC (lane 1), glomerular (lane 2), and liver (lane 3) cDNA using primers derived from mouse factor H. The 1057-bp product was identical to the sequence of rat factor H, whereas the 529-bp product was for the unique FHR product that was studied here. Size standards were run in lane 4.

**Fig. 2.** Map of full-length FHR cDNA. The three cDNA clones used to construct the full-length FHR cDNA are shown schematically in this diagram. The open reading frame is in the horizontal black bar.
FIG. 3. Nucleotide sequence of FHR cDNA and predicted protein sequence. Numbering of the nucleotide sequence is on the left and that of the predicted amino acids on the right. The signal peptide and polyadenylation signal are underlined. The two potential N-linked glycosylation sites are in bold-face type.
H (28) hybridized with a 4.3-kb band representing factor H mRNA (lane 2). To provide an estimate of the relative abundance of each mRNA, an additional Northern blot was done using the 529-bp PCR product from FHR shown in Fig. 1. As this cDNA has 91% nucleotide similarity to factor H, hybridization with this probe will detect both factor H and FHR. As shown in lane 3, both mRNA species were identified, with factor H mRNA appearing to be considerably more abundant than that for FHR.

Expression and Functional Analysis of Recombinant Rat FHR—A recombinant version of rat FHR incorporating a V5 epitope and a polyhistidine epitope tag at the C terminus was expressed in CHO-K1 cells using the pcDNA3.1/V5/His vector. Western blotting, performed under nonreducing conditions, using the monoclonal anti-His(C-terminus) antibody (lane 1), polyclonal goat anti-human factor H antibody (lanes 2 and 3), or affinity-purified anti-FHR antibody (lane 4). The sizes of the protein bands as judged by molecular weight standards run in parallel are indicated by arrows.

Fig. 4. Amino acid sequences of rat FHR, rat factor H, and human FHR-5. Where there are differences, the amino acid sequence of the respective protein is provided below that of rat FHR.

Fig. 5. Northern analysis of rat liver RNA. Total liver RNA was probed with 32P-labeled cDNA probes derived by 3′-RACE for FHR (lane 1) and factor H (lane 2). In lane 3, an FHR PCR product with high homology to factor H was used, which identified both mRNA species.

Fig. 6. Western blotting to detect FHR. Affinity-purified recombinant rat FHR (lanes 1 and 2) and rat serum (lanes 3 and 4) were electrophoresed on a 7.5% SDS-PAGE gel under nonreducing conditions, transferred to a polyvinylidene difluoride membrane, and probed using monoclonal anti-polyhistidine (C terminus) antibody (lane 1), polyclonal goat anti-human factor H antibody (lanes 2 and 3), or affinity-purified anti-FHR antibody (lane 4). The sizes of the protein bands as judged by molecular weight standards run in parallel are indicated by arrows.
combinant FHR (Fig. 6, lane 4) (the 2-kDa difference between native and recombinant protein can be attributed to the two C-terminal tags engineered in the latter). Prominent 115- and 150-kDa bands were also identified in rat sera. The latter undoubtedly represents factor H, whereas the smaller protein is likely to represent a cleavage fragment of factor H, based upon our past studies (28). This pattern was consistently observed in repeated blots. These results suggest FHR is a minor constituent of serum, at least as judged by Western blotting using a cross-reacting anti-human factor H antibody.

Because SCRs 8–15 and 19–20 in human factor H appear to be involved in binding C3b (7) and analogous SCRs are present in rat FHR, the possibility that FHR binds to rat C3b was investigated by immunooassay. As shown in Fig. 7, C3b bound to FHR in a dose-dependent and saturable manner. Thus, like with the human factor H and FHR family members, rat FHR is a C3b-binding protein.

Addition studies were performed to evaluate the capacity of FHR to regulate the alternative pathway. In these studies, factor H and FHR were immunochemically depleted from rat serum to avoid potential confounding effects on recombinant FHR function. As shown in Fig. 8, FHR inhibited spontaneous alternative pathway activation on guinea pig erythrocytes in a dose-dependent fashion. Hence, FHR also is capable of inhibiting alternative pathway activation.

Expression of Rat FHR mRNA in Rat GEC—Given the prominent appearance of FHR-5 in human membranous nephropathy (27), a disease in which complement activation occurs in the proximity of GEC, the possibility that complement activation alters the appearance of FHR mRNA was investigated. The expression of FHR was increased over time in cultured GEC exposed to anti-Fx1A, and then complement (Fig. 9, ). Under the conditions chosen for these studies, there was 83.0% release of BCECF, and there was 95.9% of maximum MTT uptake, consistent with the goals to induce injury of GEC while retaining cellular viability. This increase in FHR mRNA was specifically due to complement activation, as FHR mRNA in cells treated identically, yet exposed to heat-inactivated serum, did not change over time (Fig. 9, ).

PHN was used in these studies as a relevant model of membranous nephropathy and to study events induced by complement activation on GEC in vivo. Just as with cultured GEC exposed to complement activation by anti-Fx1A, there was a marked increase (>3.5-fold) in FHR mRNA at each time point studied in PHN (Fig. 10, ). In addition, animals in which complement was depleted with CVF had no change in FHR mRNA over time (Fig. 10, ). Thus, GEC contain FHR mRNA, and this is up-regulated in response to complement activation in vitro and in vivo.

**DISCUSSION**

During our attempt to clone factor H from cultured rat GEC, we identified the cDNA for a related protein. This protein, which we have termed FHR, contains tandemly arranged SCR modules typical for all members of the regulators of complement activation. Within this cluster of related genes is the factor H family. In humans, this family is composed of seven proteins, factor H, factor H-like protein, and FHRs 1–5. Factor H-like protein appears to arise by alternative splicing from the factor H gene, whereas the FHR proteins in humans have similarity, but not identity to factor H, and thus are presumed to be separate genes that have arisen by gene duplication (7, 37). FHRs 1–4 contain four to five SCRs, whereas FHR-5 is the longest with nine SCRs. Each of these proteins have similar C-terminal SCRs, with homology to SCRs 19–20 of factor H (7, 26, 37). Of these, human FHR-5 is most similar to rat FHR, as its nine SCRs have up to 79% homology to corresponding SCRs in rat FHR (Fig. 4). By comparison to the areas mapped in human factor H that confer binding activity for C3b, FHR-5 has two potential binding sites for C3b (8, 10), and therefore, it is not surprising that human FHR-5 has the ability to bind C3b (26). The same basic SCR structure and capacity to bind C3b is true for rat FHR, as we have shown here. In addition, we have also shown that like factor H, rat FHR is an effective regulator of the alternative pathway of complement.

The cDNAs for human factor H and the related factor H-like protein were cloned by a directed search using either nucleotide probes derived from the protein sequence or antibodies against native factor H (38–41). The next members to be cloned were FHRs 1–4 by virtue of their nucleotide similarity to the “parent” factor H (42–44). In contrast, FHR-5 was identified because it was a component of immune deposits in glomerular diseases (26). This was possible because the K2.254 monoclonal antibody raised against diseased glomeruli reacted with FHR-5 and was used to isolate FHR-5 for peptide determination, knowledge of which was then used in a cloning strategy. In these studies, it was shown that FHR-5 mRNA could be produced by the liver and that FHR-5 protein could deposit on guinea pig erythrocytes during complement activation by whole serum (26). In follow-up studies on FHR-5, it was clear that FHR-5 was a prominent component of glomerular immune deposits, being present in 100% of cases of membranous nephropathy, IgA nephropathy, and lupus nephritis (27). Its presence and distribution tracked with those of C3 and SC5b-9, the conclusion being FHR-5 was a highly sensitive marker of complement deposition. Whether it was deposited from plasma and/or produced locally could not be determined in these studies of human biopsy specimens.

In this study, we showed that cultured rat GEC contain FHR mRNA. Although not the focus of the present study, these cells also bear mRNA for factor H. This cell occupies a unique anatomic location in vivo at the distal end of a very effective filter to the passage of plasma proteins. Thus, GEC are exposed to selected proteins, those being smaller (and more positively charged) having greater access. It seems unlikely there is significant passage of the main fluid-phase complement regulators, factor H and C4-binding protein through the glomerular capillary wall, given their size (C4-binding protein is ~535-kDa) and physicochemical characteristics (for example, factor...
H behaves as a protein of 300 kDa in gel filtration, presumably because of its extended conformation (4)). Therefore, the ability of GEC to produce complement regulators locally may be necessary to prevent complement activation. For instance, although complement receptor 1 typically resides on cells of hematopoietic and immune lineage, it is also on GEC (29, 45). GEC and other glomerular cells also bear the glycosylphosphatidylinositol-linked DAF and CD59 proteins (31, 46, 47). Given its C3b binding activity, production of FHR may provide some advantage in vivo. Clearly, upon complement attack, cultured GEC respond with the production of FHR, which may represent a defense mechanism.

The distinct advantages of studying rodents is that they can be used to model human diseases, allowing studies that could never be accomplished in humans. Thus, whereas FHR-5 is a prominent component of immune deposits in membranous nephropathy, its exact role will be difficult to determine in humans. As modeled both in vitro and in vivo in the rat, FHR mRNA is this represents some form of protective response and/or is an attempt to clear the immune deposits is the subject of future studies.

Although GEC in vitro and glomeruli in vivo have the capacity to produce FHR, as judged by the presence of its mRNA, our studies show that the liver does express FHR mRNA and that FHR is a plasma protein. This is comparable with human FHR-5 (and all of the factor H family members). Thus, the appearance of FHR-5 protein in glomerular immune deposits could be simply the result of deposition of FHR-5 from plasma. Our present studies on FHR protein in the rat are limited by currently available antibodies being directed toward human factor H, which cross-react with rat factor H (28) and FHR. With the generation of specific anti-rat FHR antibodies, the potential appearance of FHR in immune deposits can be tracked in future studies.

In summary, we have identified a rat protein in the factor H family that is most similar to human FHR-5. Because it is the first FHR protein identified in the rat, we have termed it FHR. The mRNA for this protein is expressed in the liver and FHR is a component of plasma. In addition, cultured GEC and glomeruli express FHR mRNA. FHR-5 is an invariant component of immune deposits in human membranous nephropathy. As modeled both in vitro and in vivo in the rat, FHR mRNA is
dramatically up-regulated in membranous nephropathy. This is likely to be a direct response of GEC to complement attack and may represent a protective response of this cell.

REFERENCES

1. Liszewski, M. K., Farries, T. C., Lublin, D. M., Rooney, I. A., and Atkinson, J. P. (1996) Adv. Immunol. 61, 201–283
2. Campbell, R. D., Law, S. K. A., Reid, K. B. M., and Sim, R. B. (1988) Annu. Rev. Immunol. 6, 161–195
3. Nilsson, B., and M€ller-Eberhard, H. J. (1965) J. Exp. Med. 122, 277–298
4. Whaley, K., and Ruddy, S. (1976) J. Exp. Med. 144, 1147–1163
5. Whaley, K., and Ruddy, S. (1976) Science 193, 1011–1013
6. Weiler, J. M., Daha, M. R., Austen, K. F., and Fearon, D. T. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3268–3272
7. Zipfel, P. F., Jokiranta, T. S., Hellwage, J., Kaistinen, V., and Meri, S. (1999) Immunopharmacology 42, 53–60
8. Sharma, A. K., and Pangburn, M. K. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10996–11001
9. Hellwage, J., Jokiranta, T. S., Kaistinen, V., Vaarala, O., Meri, S., and Zipfel, P. F. (1999) FEBS Lett. 462, 345–352
10. Jokiranta, T. S., Hellwage, J., Kaistinen, V., Zipfel, P. F., and Meri, S. (2000) J. Biol. Chem. 275, 27657–27662
11. Soames, C. J., and Sim, R. B. (1997) Biochem. J. 326, 553–561
12. Nabi, K., Rihn, B., Jaurand, M. C., Vignaud, J. M., Ripoche, J., Martinet, Y., and Martinet, N. (1997) Biochem. J. 326, 377–383
13. DiScipio, R. G., Daffern, P. J., Schraufstatter, I. U., and Sirimamaro, P. (1998) J. Immunol. 160, 4057–4066
14. Zipfel, P. F. (2001) Semin. Thromb. Hemostasis 27, 191–199
15. Pis, E., Martinez, A., Unsworth, E. J., Kowalak, J. A., Bengoechea, J. A., Zipfel, P. F., Elasser, T. H., and Cuttitta, F. (2001) J. Biol. Chem. 276, 12292–12300
16. Couser, W. G. (1990) J. Am. Soc. Nephrol. 1, 13–29
17. Bieseker, G., Katz, S., and Koffer, D. (1981) J. Exp. Med. 154, 1779–1784
18. Schulze, M., Donadio, J. V., Jr., Prucno, C. J., Baker, P. J., Johnson, R. J., Stahl, R. A. K., Watkins, S., Martin, D. C., Wurzner, R., Getze, O., and Couser, W. G. (1991) Kidney Int. 40, 533–538
19. Brenchley, P. E., Coupes, B., Short, C. D., O'Donoghue, D. J., Balladire, F. W., and Mallick, N. P. (1992) Kidney Int. 41, 953–957
20. Kerjaschki, D., and Neale, T. J. (1996) J. Am. Soc. Nephrol. 7, 2518–2526
21. Salant, D. J., Belok, S., Madaio, M. P., and Couser, W. G. (1980) J. Clin. Invest. 66, 1339–1350
22. Cybulsky, A. V., Rennke, H. G., Feintzeg, I. D., and Salant, D. J. (1986) J. Clin. Invest. 77, 1096–1107
23. Salant, D. J., Darby, C., and Couser, W. G. (1989) J. Clin. Invest. 68, 71–81
24. Murphy, B. F., and d'Apolo, A. J. (1988) Pathology 20, 130–136
25. Murphy, B. F., Kirshazbaum, L., Walker, I. D., and d'Apolo, A. J. (1988) J. Clin. Invest. 81, 1858–1864
26. McRae, J. A., Cowan, P. J., Power, D. A., Mitchelhill, K. I., Kemp, B. E., Morgan, B. P., and Murphy, B. F. (2000) J. Biol. Chem. 276, 6747–6754
27. Murphy, B., Georgiou, T., Macht, D., Hill, P., and McRae, J. (2002) Am. J. Kidney Dis. 39, 24–27
28. Alexander, J. J., Hack, B. K., Cunningham, P. N., and Quigg, R. J. (2001) J. Biol. Chem. 276, 32129–32135
29. Quigg, R. J., Galishoff, M. L., Sneed, A. E., and Kim, D. (1993) Kidney Int. 43, 730–736
30. Quigg, R. J., Cybulsky, A. V., Jacobs, J. B., and Salant, D. J. (1988) Kidney Int. 34, 43–52
31. Quigg, R. J., Nicholson-Weller, A., Cybulsky, A. V., Badalamenti, J., and Salant, D. J. (1989) J. Immunol. 142, 877–882
32. Ren, G., Hack, B. K., Minto, A. W., Cunningham, P. N., Haas, M., and Quigg, R. J. (2002) Clin. Immunol. 103, 43–52
33. Cunningham, P. N., Hack, B. K., Ren, G., Minto, A. W., Morgan, B. P., and Quigg, R. J. (2001) Kidney Int. 60, 900–909
34. Salant, D. J., and Cybulsky, A. V. (1988) Methods Enzymol. 162, 421–461
35. Quigg, R. J., He, C., Hack, B. K., Alexander, J. J., and Morgan, B. P. (2000) Immunology 99, 46–53
36. Baede, J. J., Bergjik, E. C., Hoedemaeker, P. J., De Heer, E., and Bruijn, J. A. (1994) Nephrol. Dial. Transplant. 9, 304–308
37. Morgan, B. P., and Harris, C. L. (1999) Complement Regulatory Proteins, pp. 41–136, Academic Press, San Diego
38. Ripoche, J., Doy, A. J., Harris, T. J., and Sim, R. B. (1988) Biochem. J. 249, 593–602
39. Kristensen, T., Wetsel, R. A., and Tack, B. F. (1986) J. Immunol. 136, 3407–3411
40. Schultz, T. P., Schwabl, W., Stanley, K. K., Weiss, E., and Dierich, M. P. (1986) Eur. J. Immunol. 16, 1351–1355
41. Schwabl, W., Zwierner, J., Schulz, T. F., Linke, R. P., Dierich, M. P., and Weiss, E. H. (1987) Eur. J. Immunol. 17, 1485–1489
42. Estaller, C., Koistinen, V., Schwaeble, W., Dierich, M. P., and Weiss, E. H. (1991) J. Immunol. 146, 3190–3196
43. Skerka, C., Timmann, C., Horstmann, R. D., and Zipfel, P. P. (1992) J. Immunol. 148, 3313–3318
44. Skerka, C., Horstmann, R. D., and Zipfel, P. F. (1991) J. Biol. Chem. 266, 12015–12020
45. Kazatchkine, M. D., Fearon, D. T., Appay, M. D., Mandet, C., and Bariety, J. (1982) J. Clin. Invest. 69, 900–912
46. Quigg, R. J., Morgan, B. P., Hokers, V. M., Adler, S., Sneed, A. E., and Lo, C. F. (1995) Kidney Int. 48, 412–421
47. Quigg, R. J., Hokers, V. M., Morgan, B. P., and Sneed, A. E. (1995) J. Immunol. 154, 3437–3443