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Chemical Chaperones Curcumin and 4-Phenylbutyric Acid Improve Secretion of Mutant Factor H $R_{127}H$ by Fibroblasts from a Factor H-Deficient Patient

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Factor H (FH) is one of the most important regulatory proteins of the alternative pathway of the complement system. Patients with FH deficiency have a higher risk for development of infections and kidney diseases because of the uncontrolled activation and subsequent depletion of the central regulatory component C3 of the complement system. In this study, we investigated the consequences of the Arg^{127}His mutation in FH (FH_{R127H}) previously described in an FH-deficient patient, on the secretion of this protein by skin fibroblasts in vitro. We observed that, although the patient cells stimulated with IFN-γ were able to synthesize FH_{R127H}, the mutant protein was largely retained within the endoplasmic reticulum (ER), whereas normal human fibroblasts stimulated with IFN-γ secrete FH without retention in the ER. Moreover, the retention of FH_{R127H} provoked enlargement of ER cisterns after treatment with IFN-γ. A similar ER retention was observed in Cos-7 cells expressing the mutant FH_{R127H} protein. Despite this deficiency in secretion, we show that the FH_{R127H} mutant is capable of functioning as a cofactor in the Factor I-mediated cleavage of C3. We then evaluated whether a treatment could increase the secretion of FH, and observed that the patient's fibroblasts treated with the chemical chaperones 4-phenylbutiric acid or curcumin increased the secretion rate of FH. We propose that these chemical chaperones could be used as alternative therapeutic agents to increase FH plasma levels in FH-deficient patients caused by secretion delay of this regulatory protein. *The Journal of Immunology*, **2012, 189: 3242–3248.**

The complement system is one of the first defense barriers against microorganisms. This system consists of >30 soluble and cell-bound proteins, and can be activated by the classic, alternative, and lectin pathways (1, 2). As a result of this activation, a cascade of biochemical events is triggered with important biological consequences such as inflammation, lysis of pathogens and altered cells, clearance of immune complexes and apoptotic cells, opsonization of foreign particles for enhanced phagocytosis, binding to specific receptors on immune cell surfaces inducing cellular activation, and secretion of regulatory molecules that act in the innate and acquired immune responses (2).

The constitutive activation of the alternative pathway is responsible for most of the complement activities in the serum. This pathway is activated spontaneously after hydrolysis of a thio-ester bond located in the C3 α-chain, generating $C3(H_2O)$, which presents a binding site for Factor B (FB). Once FB is complexed to $C3(H_2O)$, Factor D cleaves FB, releasing the fragment Ba. The complex $C3(H_2O)Bb$ is the first C3-convertase of the alternative pathway and cleaves C3, generating C3a and C3b fragments. C3b can complex to FB to form C3bB, which is cleaved by Factor D to generate C3bBb, the second C3-convertase of the alternative pathway. As more C3b fragments are formed, the activation of the alternative pathway is amplified (3). To prevent exacerbated activation, which leads to complement-mediated tissue damage and wasteful consumption of complement components, the alternative pathway must be tightly regulated. One of the most important regulatory proteins is Factor H (FH). This 150-kDa protein is mainly synthesized by the liver and consists of 20 globular short consensus repeat (SCR) domains (4). FH has multiple binding regions for C3b that are located within SCRs 1–4, 12–15, and 19–20, and for heparin, located in SCR7, 9, 12–14, and 19–20 (5). Moreover, the C-terminal domain of FH contains regions for interaction with both C3b and C3d fragments with heparin and glycosaminoglycan molecules, whereas the N-terminal region (SCRs 1–4) of the molecule is responsible for complement regulatory activity (5) because it binds preferably to C3b fragments.

FH regulates the alternative pathway by competing with FB for C3b binding, preventing the formation of the alternative pathway C3-convertase and attenuating C3 consumption on self-cells. Moreover, FH is an important cofactor of Factor I (FI), and its ability to cleave C3b and generate iC3b on the surface of pathogens (2). The modulation of these functions requires the recognition of cell membrane components such as sialic acid and polyanions (6, 7).

Patients with FH deficiency are more susceptible to recurrent infections as a consequence of diminished C3 levels because of its uncontrolled consumption. They are also prone to develop membranoproliferative glomerulonephritis type II (MPGN II) because they have defective complement regulation in the kidney (8, 9). We have previously characterized the FH deficiency in a child who

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Abbreviations used in this article: ER, endoplasmic reticulum; FB, Factor B; FH, Factor H; FH_{R127H}, Arg^{127}His mutation in FH; FI, Factor I; MPGN II, membranoproliferative glomerulonephritis type II; PBA, 4-phenylbutyric acid; PDB, Protein Data Bank; SCR, short consensus repeat.

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presented a homozygous single nucleotide substitution (CG^{453}T to CA^{453}T) leading to an R_{127}H substitution in the FH protein (10). This patient presented undetectable complement-mediated hemolytic activity, undetectable levels of C3 and FB, and very low serum levels of FH (10). Furthermore, the mutant Arg^{127}His mutation in FH (FH_{R127H}) is retained intracellularly and slowly secreted to the cell culture supernatant (10). In this study, we investigated the mechanisms by which this mutation causes deficiency of this protein in the patient's serum. Our results show that FH_{R127H} is retained in the endoplasmic reticulum (ER) of patient fibroblasts, and we present evidence that chemical chaperones may aid to stabilize the mutant protein and consequently restore its ability to be secreted. These chaperones may therefore represent potential therapeutic agents in the treatment of FH-deficient patients carrying this lesion or lesions with similar effects on FH stability.

Materials and Methods

Cell culture and reagents

Primary human fibroblasts were isolated by skin biopsy as described previously (11) and modified later (12). The Institutional Review Board approved the project, and informed consent was obtained from the patient's family. Cos-7 cells were purchased from the American Tissue Culture Collection. Primary fibroblast and Cos-7 cell cultures were maintained in DMEM supplemented with 10% heat-inactivated FCS (Invitrogen Life Technologies) and 100 U/ml penicillin, 100 μg/ml streptomycin (Bio-Lab), and 2 mM L-glutamine (Invitrogen Life Technologies).

Cell culture treatments

Patient fibroblast cultures were treated with 1 and 2 μM curcumin (Sigma-Aldrich) dissolved in DMSO or with 1 and 2 mM 4-phenylbutyric acid (PBA; Sigma-Aldrich) dissolved in Milli-Q H_2O for up to 24 h.

Expression of FH by transfected Cos-7 cells

PCR-based, site-directed mutagenesis (QuickChange II Site-Directed Mutagenesis kit; Stratagene) was used to introduce the CA^{453}T substitution in the FH cDNA contained within the mammalian expression vector pIC-neo (Promega) kindly provided by Dr. Santiago de Córdoba from Centro de Investigaciones Biológicas, Conseio Superior de Investigaciones Científicas, Madrid, Spain (13). The following mutagenic primers were used (the altered bases are highlighted in bold): 5′-GCTAGGTGAGATTAATTACC**A**TGAATGTGACACAGATGGATG-3′ and 5′-CATCCATCTGTGTCACATTCA**T**GGTAATTAATCTCACCTAGC-3′. Cos-7 cells (1.5×10^5 cells/well) were transfected with 650–750 ng pIC-Neo containing wild-type (C**G**^{453}T) or mutant (C**A**^{453}T) FH cDNA, using 3 μl lipofectamine 2000 (Invitrogen Life Technologies) according to the manufacturer's protocol. The clones were selected using DMEM containing G418 (1 mg/ml) for 7 d, and FH production was evaluated by immunofluorescence.

Analysis of FH secretion by Western blotting

Skin fibroblasts (10^6 cells) from the patient and a normal control were cultured and stimulated with 100 U/ml IFN-γ (R&D Systems) for 6, 12, 24, and 48 h. This cytokine enhances the expression of FH by fibroblasts (14). After incubation, we separated the cells and the cell culture supernatants. The cells were suspended in 100 μl lysis buffer (PBS 1X, 10 mM EDTA, 2mM phenylmethanesulfonylfluoride, 1% Triton X-100 and protease inhibitor mixture; GE Healthcare). After one cycle of freezing and thawing, cell lysates were subjected to centrifugation for 5 min at $10,000 \times g$ and the supernatants were harvested for further analysis. The supernatants were concentrated 10 times by lyophilization. The protein concentration of each sample was determined using Bradford's protein assay, and equivalent amounts of protein were separated by SDS-PAGE. After transfer onto a nitrocellulose membrane, nonspecific sites were blocked with TBS-Tween buffer (5 mM Tris-HCl [pH 8.0], 75 mM NaCl, 0.028% Tween-20) containing 5% nonfat dried milk, for 1 h at room temperature. The membrane was then treated with rabbit anti-human FH Ab (1:10,000; kindly provided by Dr. Pilar Sánchez, Unidad de Inmunología, Hospital Universitario La Paz, Madrid, Spain) for 2 h at room temperature. The membrane was then incubated with an HRP-conjugated secondary Ab (1:10,000; Sigma-Aldrich) for 1 h at room temperature. Bands were visualized with an ECL reagent (Pierce) after exposition of Hyperfilm ECL (GE Healthcare). Western blot analysis to evaluate FH secretion by transfected Cos-7 cells was carried out using goat anti-human FH (1:10,000; Quidel) as primary Ab using the procedure described earlier.

Immunofluorescence microscopy

Human fibroblasts were stimulated with 100 U/ml IFN-γ for 24 h and prepared for confocal microscopy immunofluorescence as described previously (10). Cells were labeled with rabbit anti-human FH (kindly provided by Dr. Peter Zipfel, Department of Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena, Germany). To determine the intracellular location of the mutant FH protein within the patient's fibroblasts, we also used the following Abs: mouse anti-calnexin (ER marker; BD Bioscience), mouse anti–α-mannosidase (Golgi complex marker), and mouse anti-β COP (ER, Golgi complex, and vesicles from Golgi complex marker), kindly provided by Dr. Antonio Sesso (Faculty of Medicine, University of São Paulo, São Paulo, Brazil). We also used DAPI for nuclei labeling. Anti-IgG secondary Abs were conjugated with FITC or tetramethylrhodamine isothiocyanate (Sigma-Aldrich). The analyses were performed using a confocal microscopy (ZEISS LSM 510 Meta).

Transmission electron microscopy

For electron microscopy, 5×10^5 human fibroblasts were cultured with 100 U/ml IFN-γ for 48 h. Cells were then harvested by centrifugation at $750 \times g$ for 5 min and washed in PBS. The cells were fixed with 4% formaldehyde, 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.3, for 24 h at 4°C. After fixation, the cells were centrifuged at $750 \times g$ for 5 min, and the pellet was washed twice and postfixed for 2 h with 1% osmium tetroxide and 0.8% potassium ferrocyanide in 0.2 M sodium cacodylate buffer. The pellet was then washed and incubated with 0.5% uranyl acetate, 10% sucrose solution for 24 h at 4°C. Finally, the pellet was dehydrated through an increasing alcohol series (70–100%), infiltrated with propylene oxide plus resin (v/v) for 12 h, and embedded in Spurr resin. Ultra-thin sections were observed in a transmission electron microscopy (JEOL 100C-XL).

FH cofactor activity assay

FH protein was purified by immunoaffinity chromatography from supernatants of patient and control fibroblast cultures. We incubated 250 ng C3b, 100 ng FI (Complement Technology), and 100–200 ng purified FH in PBS solution (final volume 50 μl) for 3 h at 37°C. An aliquot of $4 \times$ reducing SDS buffer (250 mM Tris-HCl 1M pH 6.8, 10% SDS, 0.5% bromophenol blue, 50% glycerol, 7% 2-ME) was then added to the samples and heated for 5 min at 95°C before loading into SDS-PAGE. The FH cofactor activity was confirmed after visualization of an ~43-kDa band corresponding to iC3b by Western blotting.

Results

FH secretion analysis

To determine whether cells from the deficient patient are able to synthesize and secrete the FH_{R127H} mutant, we stimulated skin fibroblasts from this patient and a normal individual with IFN-γ for 6, 12, 24, and 48 h, and analyzed the cellular and secreted FH by Western blotting. FH molecules with the expected size (150 kDa; Fig. 1A) could be detected in fibroblast cultures from both patient and control cells. However, the patient and control cells presented significant differences in the temporal evolution of their FH secretion ratios. Although normal cells secrete wild-type FH in <6 h after synthesis and do not accumulate significant amounts intracellularly, patient fibroblasts retain the mutant protein intracellularly, and the mutant FH is observed in the culture supernatants only 48 h after IFN-γ treatment (Fig. 1A). To determine whether the observed delay in FH secretion by patient fibroblasts is due to the FH_{R127H} mutation itself or to some other factor in the deficient patient cells, we transfected Cos-7 cells with pIC-neo-FH C**G**^{453}T (wild-type) and pIC-Neo FH C**A**^{453}T (mutant) plasmids. Fig. 1B shows that extracellular wild-type FH levels are always greater than the intracellular levels, whereas the intracellular levels of the mutant FH protein are greater than the extracellular

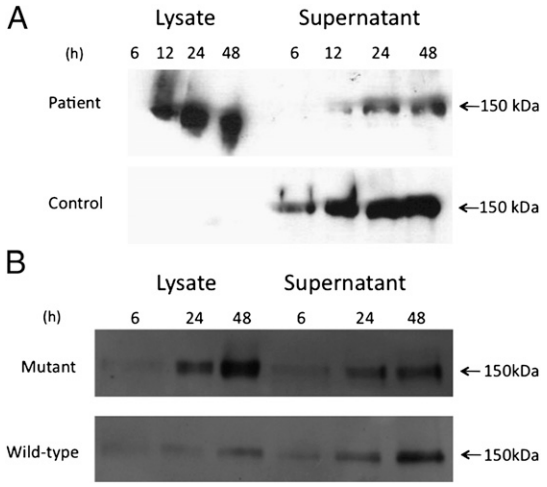

FIGURE 1. Secretion ratio of FH by fibroblasts. (**A**) Human fibroblasts were treated with IFN-γ. (**B**) Cos-7 cells were transfected with pIC-neo-FH $CG^{453}T$ (mutant) or pIC-neo-FH $CA^{453}T$ (wild-type). After incubation, the cell lysates and supernatants were harvested, concentrated, and equal amounts of protein were loaded in SDS gel, followed by Western blot analysis using anti-human FH.

levels at all times. These results confirm that the $R_{127}H$ mutation does, in fact, reduce the efficiency of FH secretion by patient fibroblasts.

FH_{R127H} presents normal regulatory activity

Considering the low levels of mutant FH_{R127H} in the patient serum and the slow kinetics of FH secretion by patient fibroblasts, we asked whether the mutant protein could carry out specific functions attributed to FH in the regulation of the alternative pathway of the complement system. To approach this question, we first incubated patient and normal control serum with purified C3b and FI for 3 h at 37˚C. Under these conditions, both sera cleaved C3b, generating an ∼43-kDa band, which corresponds to one of iC3b α′-chains (Fig. 2A). The 67-kDa band from iC3b α′-chain was not observed probably because it comigrates with the iC3b β-chain (70 kDa). To confirm this result, we asked whether purified mutant FH_{R127H} was able to cleave C3b into iC3b. We therefore obtained wild-type and mutant FH molecules from skin fibroblast culture supernatants or from transfected Cos-7 cell supernatants by purification using anti-FH linked immunoaffinity chromatography. Equal amounts of purified wild-type FH or mutant FH_{R127H} proteins were incubated with commercial C3b and FI for 3 h at 37˚C. We observed that the mutant protein was able to cleave C3b into iC3b with efficiency equal to that of wild-type FH (Fig. 2B). Therefore, both wild-type FH and mutant FH_{R127H} proteins are able to act as a cofactor of FI in the cleavage of C3b.

Intracellular localization of mutant protein FH_{R127H}

The observed delay in secretion of the mutant FH_{R127H} raised the question as to in which intracellular compartment this mutant protein is retained in the patient's cells. To address this question, we stimulated patient's fibroblast cultures with IFN-γ for 24 h and then labeled with anti-FH Ab for immunofluorescence analysis. Fibroblasts from the FH-deficient patient presented more intense intracellular labeling than fibroblasts from the normal control (Fig. 3A). This result is consistent with the delayed secretion and intracellular accumulation of mutant FH_{R127H} observed after Western blotting analysis shown in Fig. 1. Furthermore, we observed a diffuse reticular staining of the cytoplasm especially prominent in the perinuclear region (Fig. 3A), suggesting that mutant FH_{R127H} could be retained near the Golgi complex. We observed that mutant FH_{R127H} colocalizes with β-COP, a well-known marker of ER and Golgi complex (Fig. 3A). These results suggest that FH_{R127H} is retained within these compartments.

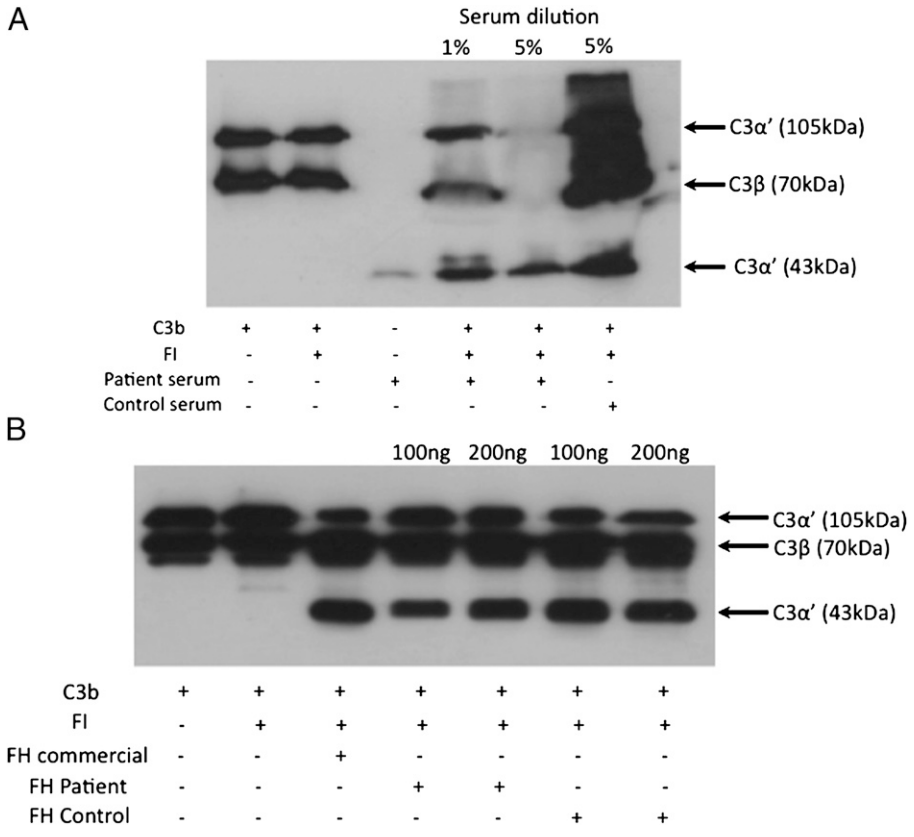

FIGURE 2. Evaluation of cofactor activity of mutant FH. (**A**) Purified commercial C3b and FI were incubated with 1 or 5% serum for 3 h. (**B**) C3b and FI were incubated with 100 or 200 ng FH purified from supernatant of patient's and control's fibroblasts for 3 h. FH cofactor activity was evaluated by the presence of the C3b cleavage product (C3 α′-chain fragment, 43 kDa).

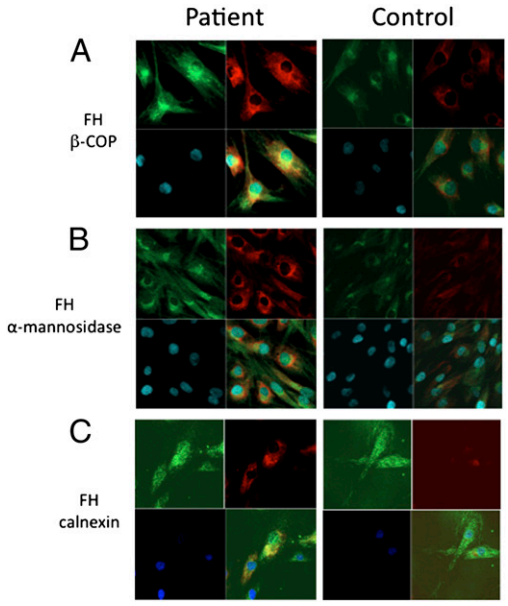

FIGURE 3. Intracellular localization of mutant FH in fibroblasts treated with IFN-γ, by immunofluorescence microscopy. The cells were fixed and labeled with Ab against specific proteins. Green represents anti–β-COP (**A**), anti–α-mannosidase (**B**), or anti-calnexin (**C**); red represents FH; blue represents DAPI (nuclei); yellow represents merge. All images were obtained with a plan-apochromat 63×/1.4 oil differential interference contrast objective.

To investigate this question further, we labeled the patient fibroblasts with anti-FH, with anti–α-mannosidase Ab specific to the Golgi complex, and with anti-calnexin Ab specific for the ER. Moderate colocalization of FH_{R127H} and anti–α-mannosidase was observed. The greater part of FH_{R127H} staining was observed outside the Golgi complex, suggesting another compartment where the mutant protein was retained in the patient cells (Fig. 3B). In double-staining experiments using anti-FH and anti-calnexin, we observed colocalization between FH and calnexin, confirming that the protein is retained in the ER (Fig. 3C).

We then asked whether mutant FH retention resulted in other intracellular modifications. To address this, we used electronic microscopy to analyze patient and control fibroblasts after treatment with IFN-γ for 48 h. We observed that the patient's fibroblasts presented larger ER cisterns than control fibroblasts (Fig. 4). This modification in ER structure has been associated with the response to the accumulation of unfolded proteins during ER stress.

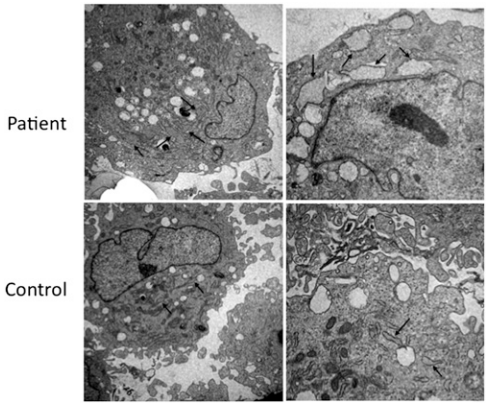

FIGURE 4. ER integrity in fibroblast's culture from patient and control. Black arrow indicates the ER in fibroblasts treated with IFN-γ for 48 h. Original magnification ×5000 (*left panels*), ×10,000 (*right panels*).

Evaluation of the secretion by fibroblasts of FH patients treated with chemical chaperones

Because the mutant FH_{R127H} protein has regulatory activity but may be causing ER stress, we sought to improve FH secretion by treating the patient's cells with drugs that facilitate protein folding. Different investigators have used chemical chaperones, such as curcumin and PBA, to improve the secretion of unfolded proteins (15–18). We therefore treated patient's fibroblasts with increasing doses of curcumin and PBA for 24 h. Both drugs improved the in vitro secretion of mutant FH_{R127H} by fibroblasts after 24 h treatment even in the absence of IFN-γ. Concentration–response analysis of FH secretion in response to chemical chaperone treatment showed that the effect in the FH secretion was maximal at a curcumin concentration of 2 μM and a PBA concentration of 2 mM (Fig. 5A). In addition, we noted that the effect of these chaperones persists for at least 12 h posttreatment for both drugs (Fig. 5B, 5C). However, the maximal effect was observed within 6 h after treatment. Western blot analysis showed that the effect of curcumin lasts longer than that of PBA because the corresponding 150-kDa band was still intense even after 24 h.

Discussion

Complete FH deficiency (homozygous) is rare: only 23 cases in 13 different families have been reported in the literature (8, 9). Patients with absence or dysfunction of FH are more susceptible to bacterial infections and development of MPGN II. This latter disease is associated with abnormalities of complement regulation and can be observed in patients carrying homozygous or heterozygous FH deficiencies. These patients exhibit mutations located in different regions of this protein such as $Cys^{518}Arg$, $Cys^{941}Tyr$ (19), $Arg^{127}Leu$, $Cys^{431}Ser$, $Cys^{673}Ser$, $Cys^{673}Tyr$ (8), ΔLys^{224} (20), $Pro^{139}Ser$ (21). All these mutations are associated with secretion delay of FH.

Our laboratory characterized a case of FH deficiency in a male child with several episodes of pneumonia. Low levels of FH (16.8 μg/ml; reference range: 225–1636 μg/ml) and undetectable levels of C3 and FB were observed in his serum, confirming an immunochemical profile compatible with FH deficiency (10) because the loop of amplification of the alternative pathway is uncontrolled. The patient carries a mutation in his FH gene that results in an $R^{127}H$ substitution in FH SCR2 (10). An equivalent mutation was previously observed in two brothers carrying MPGN II, but it was not characterized further (8).

In this study, we explored the consequences of the FH_{R127H} mutation on FH production by the patient cells. The results showed that even though the patient is able to synthesize FH protein, most of the mutant protein is retained intracellularly in the patient's fibroblasts. We observed a similar secretion delay when in transfected Cos-7 cells expressing the mutant protein. Therefore, this impaired FH secretion is not caused by an intrinsic defect in the patient's fibroblasts, and it is a consequence of the $R^{127}H$ substitution in the FH molecule.

FH_{R127H} is retained in intracellular compartments of the secretory pathway, which includes Golgi complex and ER as observed when patient's fibroblasts were labeled with anti–β-COP and anti-FH. Apparently, mutant FH is retained mainly in the ER because only a slight colocalization between α-mannosidase and mutant FH was observed in the patient's fibroblasts. The relative importance of the ER compartment for the retention of this FH mutant protein was confirmed after labeling patient's cells with anti-calnexin and anti-FH. These results together suggest two

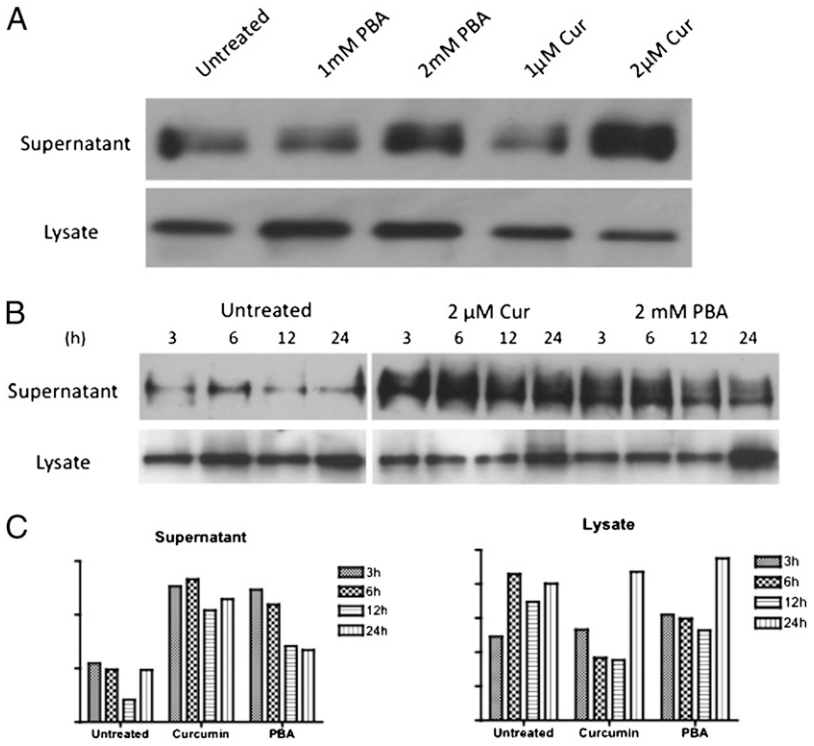

FIGURE 5. Effect of curcumin and PBA treatments on FH secretion by patient's fibroblasts. (**A**) Patient's fibroblasts were treated with 1 and 2 mM PBA or 1 and 2 μM curcumin (Cur) for 24 h. (**B**) Patient's fibroblasts were treated with 2 μM curcumin or 2 mM PBA for 3 up to 24 h. Cell lysates and supernatants were subjected to Western blotting analysis with anti-FH and HRP-conjugated secondary Ab, and the relative densitometry analysis (**C**) was performed using the software ImageJ and expressed in arbitrary units. The possibility that DMSO (used to dissolve curcumin) could be responsible for the observed increase in the FH secretion by patient's fibroblasts treated with curcumin was discarded because the effect was curcumin dose dependent.

possibilities: 1) colocalization observed in the Golgi complex is caused by the accumulation of mutant protein molecules that have escaped from the ER quality-control system and are transported to a region known as the intermediate compartment of the ER–Golgi complex. In this case, the unfolded protein cannot be released into the Golgi complex and eventually returns to the ER, where it is refolded or degraded (22); and 2) considering that the patient can secrete low levels of FH, colocalization could be explained by the presence of proteins in the process of being secreted.

A large fraction of the mutant FH protein is retained in the ER, which may cause an abnormal accumulation of this molecule and provoke enlargement of ER cisterns after 48 h treatment with IFN-γ. This event is characteristic of protein retention and causes ER stress. Enlarged ER cisterns have been observed by other groups: Truettner et al. (23) induced brain ischemia in rats and observed the accumulation of unfolded and aggregated proteins in the cytoplasm, mitochondria, and ER. In another study, protein accumulation and ER stress response were observed in patients with p31 deficiency, a protein implicated in the ER organization (24).

The association between FH deficiency and protein retention at the ER has been reported previously. Ault et al. (19) described an FH-deficient patient who developed MPGN II. This patient was able to synthesize FH protein, but it was retained in the ER. FH in this patient had two substitutions ($Cys^{518}Arg$ and $Cys^{914}Tyr$) that disrupt disulfide bonds essential for the formation of "sushi" domains SCR9 and SCR16 (19, 25). However, the functionality of this protein was not tested. These substitutions are chemically more drastic than the R127H substitution, which exchanges one basic amino acid for another in our patient. This could explain, in part, the low levels of serum FH.

Three high-resolution structures of human FH fragments containing SCR2 have been deposited in the Protein Data Bank (PDB): the NMR solution structures of SCR 1–2 and SCR 2–3 (PDB ID: 2RLP and 2RLQ, respectively) (26), and the crystal structure of SCR 1–4 in complex with complement fragment C3b (PDB ID: 2WII) (27). In the crystal structure, the Arg127 side chain projects from the internal face of a three-stranded antiparallel β-sheet toward the opposite surface of the SCR2 so that the aliphatic portion of the side chain is completely buried, whereas the guanidino group is solvent exposed (Fig. 6). The ensemble of solution structures shows that the burial of the guanidino group can vary from highly solvent exposed to almost completely buried (26). This suggests that SCR2 structure may be sufficiently flexible to accommodate the sterically different histidine side chain in the mutant protein without compromising its topology and regulatory functionality. The substitution may, however, affect the kinetics of protein folding leading to its retention the ER (15–18).

Although the $R_{127}H$ mutation is responsible for the FH secretion delay, we demonstrated that the mutant protein is able to regulate the alternative pathway of the complement system. Because the mutant protein is functional and the cells are able to secrete a small fraction of the FH produced, albeit at a lower rate, we decided to try to improve FH secretion using drugs required for protein folding. Chaperones and folding enzymes act to avoid

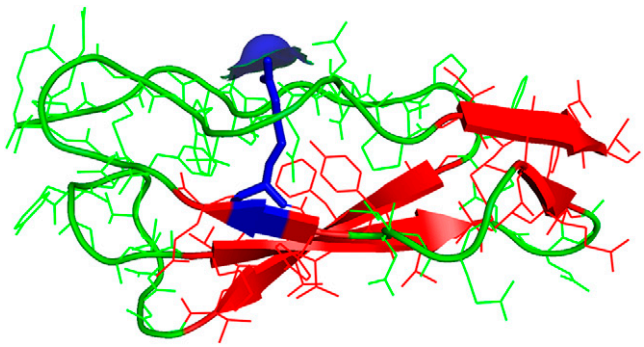

FIGURE 6. Model of the FH SCR2 domain highlighting residue Arg^{127} (blue stick model). The exposed surface of the Arg^{127} guanidino group is shown as a semitransparent blue surface. β-Strand residues are shown in red, and random coil residues are shown in green. The model was derived from FH residues 83–143 of the crystal structure of the C3b–FH complex (PDB ID: 2wII) (27). The figure was produced using the Pymol molecular graphics software.

protein aggregation and recover misfolded proteins. Although most chaperones studied to date are proteins, chemical chaperones are small molecules that exhibit similar effects within the cell. For example, pharmacological treatment with curcumin and PBA has been explored by several groups to release unfolded proteins and diminish the ER damage (15–18). Chemical chaperones may therefore be a promise to treat certain diseases associated with protein misfolding. When we treated cultures of patient's fibroblasts with curcumin or PBA, we observed that both drugs accelerate the secretion ratio of the mutant protein in fibroblast cultures, and that this beneficial effect persisted for at least 6 h.

The effectiveness of chemical chaperones depends on the chemical nature of the mutation and its structural consequences in the target protein (15). In the case of frameshift mutations, substitutions of specific amino acids (such as Cys, Tyr, and Pro) or amino acids with important structural functions, these drugs could be of limited benefit (15). Chemical chaperones are good candidates to experimentally improve the secretion of FH in deficient patients caused by mutations where relatively mild structural perturbations do not compromise protein activity, but rather reduce the secretion ratio of this important regulatory protein of the complement alternative pathway.

Although both curcumin and PBA are able to increase extracellular levels of FH in vitro, curcumin was shown to be more effective because its effects last for a longer period of time than that of PBA. Therefore, it would be the best choice for future clinical trials because it is found in several natural food products and used as dietary supplement in some countries (28–31). Furthermore, the maximum nontoxic dose of curcuminoids in humans (12 g/d) is much higher than the dose used in oral treatment (2–8 g/d) (29, 30, 32). However, curcumin consumption can produce adverse effects such as headaches, stomach ache, rashes, and diarrhea depending on the patient's sensitivity to the drug (29, 30, 32).

Curcumin is able to decrease the secretion of proinflammatory cytokines such as IL-1β and IL-6 in PBMCs of patients with chronic kidney disease (31). Thus, curcumin could act to decrease inflammation triggered by complement activated fragments, reducing tissue damage in deficient patients with delay of FH secretion. Treatment with curcumin could increase the levels of serum FH in those cases in which the deficiency is caused by defects in protein folding. Increased FH levels would restore regulation of the alternative pathway of complement system and consequently reduce the incidence of infections, besides acting to delay disease progression in MPGN II. Thus, the use of curcumin could be tested as an alternative treatment to improve FH-deficient patients' survival carrying the FH_{R127H} mutation (10) and related mutations such as FH_{R127L} reported by Dragon-Durey et al. (8).

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Disclosures

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