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Mutations of Complement Factor I and Potential Mechanisms of Neuroinflammation in Acute Hemorrhagic Leukoencephalitis

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Abstract

Purpose—Acute Hemorrhagic Leukoencephalitis (AHLE) is a rare demyelinating disorder of acute onset, rapid deterioration and significant morbidity and mortality. Most often described as a post-infectious complication of an upper respiratory illness, its precise pathophysiology remains unclear. We describe two pediatric patients with AHLE with partial complement factor I (FI) deficiency whose successful treatment included the interleukin-1 (IL-1) receptor antagonist, anakinra, implicating a role for FI and IL-1 in this disorder.

Methods—Extensive clinical workup of two patients presenting with AHLE revealed complement abnormalities, specifically related to the alternative pathway and its regulator, FI. Aggressive management with steroids, immunoglobulin, and anakinra ultimately led to improvement of clinical status and near return to neurologic baseline in both patients. Genetic sequencing of the FI coding regions of the patients and their families was performed. *In vitro* protein expression studies and immunohistochemistry of fixed brain tissue was used to investigate pathogenic mechanisms.

Results—Two novel mutations in FI were identified in our patients, which result in failure to secrete FI. Immunohistochemical evaluation of brain tissue demonstrated positive staining for C3, membrane attack complex (MAC) and IL-1.

Conclusions—We propose AHLE is an unreported, rare phenotype for partial FI deficiency. The upregulation of C3, MAC and IL-1 with subsequent demyelination support a pathologic role for complement activation in AHLE, and suggest anakinra as an important adjunctive therapy in this disease.

Keywords

Acute Hemorrhagic Leukoencephalitis; Complement Factor I; IL-1; IL-1 receptor antagonist

INTRODUCTION

Acute Hemorrhagic Leukoencephalitis (AHLE) is a rare disorder characterized by rapid neurologic decline, peripheral and cerebrospinal neutrophilia, with demyelinating disease evident on neuroimaging. Pathologic features of perivascular neutrophilic and macrophage inflammation and focal demyelination are observed on brain biopsy or postmortem examination [1]. Currently, the etiology of AHLE remains unknown, although it has been associated with upper respiratory infections, mumps, and *Mycoplasma pneumoniae*. Untreated disease is uniformly fatal. Survival has been reported in rare pediatric cases of AHLE in which early treatment was implemented, including decompressive craniotomy and immunomodulatory therapies such as intravenous immunoglobulin (IVIG) and high dose intravenous corticosteroids [1, 2].

In this report, we describe two pediatric patients with AHLE, in whom partial FI deficiency was identified. Both patients were treated with corticosteroids, IVIG, and hypertonic saline [3] with limited clinical response, but demonstrated dramatic improvement when the interleukin-1 (IL-1) receptor antagonist, anakinra, was added to the treatment regimen. These cases suggest a role for complement and IL-1 in the pathogenesis of AHLE.

MATERIALS & METHODS

Patient Samples and Genetic Sequencing

Blood samples were obtained from the two patients described as well as their immediate family members. All participants, or parents or legal guardians if the patient was a minor child, provided written informed consent under protocols approved by the UCSD institutional review board and have been performed in accordance with the 1964 Declaration of Helsinki and its later amendments. The thirteen exons of *CFI* were PCR amplified and sequenced to identify coding and splice site mutations using previously described primers [4].

Measurement of serum complement Factor I

Factor I was measured by radial immunodiffusion using an in-house assay developed at National Jewish Health, Denver, CO. The 2 standard deviation range is 29.3 – 58.5 µg/mL based on 30 healthy adults. The data for the range has normal distribution and the interassay variability is 7.7% for 65 specimens.

Expression of recombinant Factor I proteins

A pcDNA3-based expression vector for full-length human FI with a six-histidine tag at the N-terminus of the mature protein [5] was obtained as a generous gift from Dr. Sara C. Nilsson and Dr. Anna M. Blom (Lund University, Malmö, Sweden). Patient mutations in *CFI*, G212T and G587C, were introduced using a QuikChange protocol as per manufacturer's instructions with the following primers, CTA CCG TAT CAG TGC CCA AAG AAT GTC ACT GCA GTG TGT GCA ACT AAC AGG and GGA TTA GAG ACC AGT TTG GCT GAA TCT ACT TTT ACT AAG AGA AGA ACT ATG, and their complements respectively. The *CFI* coding sequence in each of the mutated plasmids was verified by sequencing (Retrogen, Inc., San Diego, CA). Plasmids containing either wild-type *CFI* or one of the mutations were separately transiently transfected in HEK293 cells [6]. Protein synthesis and transfection efficiency were evaluated by flow cytometry. In these experiments, transfected cells were incubated for 4 hours with Brefeldin A (10 µg/mL) to maximize detection of FI intracellularly. Cells were permeabilized and stained with a murine monoclonal antibody to the six-histidine tag (Roche, Indianapolis, IN) followed by FITC-labeled polyclonal rat anti-mouse antibody (eBioscience, San Diego, CA). A second plasmid containing the *IL-6* gene was used as a control for intracellular analysis of a secreted protein. Events were collected using the BD LSR II and FACSDiva software (BD Biosciences) with data analysis using Flow-Jo software. For measurement of protein secretion, supernatants from transiently transfected cells were collected after 24 hours, concentrated over Amicon Centrifugal Filters 10K (Millipore), and bound to Ni-NTA beads (Qiagen, Valencia, CA). Protein was eluted from the beads by serial transfers to buffers containing 0.5M NaCl, 20mM Tris, 10% glycerol, 2mM DTT, 0.1% NP40, EDTA-free mini-complete protease inhibitors (Roche, Indianapolis, IN) and imidazole ranging from 0 mM to 600 mM. Protein expression was determined by Western blots using a mouse monoclonal antibody to the six-histidine tag (Roche Applied Science, Indianapolis, IN). The volume of each fraction loaded was normalized to the absorbance at 280 nm.

Immunohistochemistry

Immunohistochemistry on formalin-fixed, paraffin specimens was performed with rabbit polyclonal anti-human C3c (1:200, DAKO, Carpinteria, CA), murine monoclonal anti-human MAC (1:100, Quidel, San Diego, CA) and murine anti-human IL-1 (1:100, R and D Systems, Minneapolis, MN) using the Biocare Medical Mach 4 Detection System (Biocare Medical, Concord, CA) by modifications of previously published protocols as follows [7, 8].

Briefly, antigen retrieval was performed with Biocare Medical Borg Decloaker (Biocare Medical, Concord, CA). Protease digestion with Proteinase K (Biocare Medical, Concord, CA) for 5 minutes following antigen retrieval was required to completely uncover the C3c epitope [8, 9]. Sections from renal biopsies of patients with lupus glomerulonephritis and sections of normal brain biopsies were used as positive and negative controls, respectively.

RESULTS

Patients

Patient A is a 17 year-old female of Filipino descent, who at 10 years of age developed diffuse cerebral vasogenic edema and cerebrospinal fluid (CSF) pleocytosis of neutrophils and red blood cells [2]. She had been previously healthy, and immunizations were up to date. She had an unremarkable course of community-acquired varicella zoster infection several years prior to admission, and there were no other illnesses to suggest an underlying immunodeficiency. Partial hemicraniectomy for an evolving herniation syndrome was performed and brain biopsy obtained. Histopathology revealed perivascular hemorrhagic necrosis, subacute inflammation in the subcortical white matter, patchy demyelination and an inflammatory infiltrate with a neutrophilic and histiocytic predominance [2]. After an infectious etiology was ruled out, she was treated with high-dose corticosteroids and IVIG, as previously described. She recovered with only a mild learning disability, and remained healthy for seven years.

At the age of 17, she suffered a relapse with a similar presentation of headache and hallucinations and was re-admitted to Rady Children's Hospital, San Diego. In the interim, there were no additional infections, aside from mild upper respiratory infections. On admission, she was afebrile, and her physical exam was notable for nystagmus, reduced pupillary reaction to light and an unsteady gait. MRI of her brain showed an interval development of diffuse white matter edema of the cerebrum and cerebellum (Figure 1a). CSF revealed pleocytosis with 392 WBC/ μ L of which 73% were neutrophils, and 665 RBC/ μ L. The concentration of myelin basic protein was >800 ng/mL (normal <1.5 ng/mL). In the CSF, no oligoclonal bands were seen and a source of acute infection could not be identified (Supplemental Table 1).

Despite immediate treatment with high-dose corticosteroids, IVIG, and hypertonic saline for elevated intracranial pressure [3], she developed declining mental status with status epilepticus. In the absence of detectable infection, CSF neutrophilia and neutrophilic brain infiltration suggested an innate immune basis for the injury. Our previous experience with IL-1 receptor antagonist (IL-1Ra) responsive neutrophilic inflammation in autoinflammatory diseases [10] and evidence that the recombinant form of IL-1Ra, anakinra, is effective in reducing CNS-inflammation in patients with neonatal onset multisystem inflammatory disease (NOMID) [11], suggested that this might be an effective adjunctive therapy. Treatment with anakinra was initiated at 100 mg given daily subcutaneously, concurrent with corticosteroids. Within weeks, the patient recovered her pre-admission neurologic status with marked improvement of her MRI findings (Figure 1b). Prednisone and anakinra were tapered over six and eight months, respectively, with no emergence of relapse in 21 months of follow-up.

Extensive laboratory evaluation of autoimmune and inflammatory parameters was pursued upon her second AHLE presentation, demonstrating a negative ANA, and absence of C3 nephritic factor, phospholipid antibodies, anti-dsDNA, anti-SSA and anti-SSB antibodies (Supplemental Table 1). Her only significant findings were complement abnormalities with a C3 of 41 mg/dL (normal range 74–127 mg/dL), a C4 of 30 mg/dL (18–47 mg/dL), CH50 of 21 U/mL (30–75 U/mL) and AH50 of $<10\%$ of normal (75–170). Further evaluation of

the alternative pathway demonstrated Factor B of 43.8 µg/mL (127.6–278.5 µg/mL), Factor D of 1.63 µg/mL (1.69–3.08 µg/mL), properdin 20.2 µg/mL (22.3–67.6 µg/mL), Factor I of 4.6 µg/mL (29–59 µg/mL), and Factor H of 107 µg/mL (160–412 µg/mL) consistent with rapid turnover of the alternative pathway. Complement studies on immediate family members revealed low FI levels in the healthy sister. Her mother, father and brother, also healthy, were found to have mildly reduced FI levels (Figure 2).

Patient B is a 10 year-old, unrelated male of Filipino descent who presented to Rady Children's Hospital, San Diego with headache and fatigue, with rapid neurologic decline over several hours. He was previously healthy, and had received all recommended immunizations for his age. His past medical history, as related to infections, was unremarkable except for mild upper respiratory infections and there were no prior illnesses suggestive of immunodeficiency. There was a history of an influenza-like illness three weeks prior to presentation. On admission to the ICU, he was afebrile, and physical exam was notable for difficulties with expressive language and decreased strength. Primary evaluation demonstrated a peripheral leukocytosis of 20.9×10^3 cells/µL, with 91% segmented neutrophils and elevated inflammatory markers, including CRP and ESR (Supplemental Table 1). Examination of the CSF demonstrated a significant pleocytosis (607 cells/µL) of predominantly neutrophils (91%) and myelin basic protein >900 ng/mL. Initial CT imaging of the brain demonstrated vasogenic edema requiring a left posterior craniotomy and MRI revealed the extensive white matter signal abnormalities in the corpus callosum and left temporoparietooccipital lobe consistent with encephalitis (Figure 1c).

Histopathologic examination of brain biopsy tissue revealed neutrophilic inflammation with multiple foci of myelin degradation, predominantly surrounding the vascular structures, with reactive endothelial cell changes and no distinct evidence of vasculitis (Figure 1f). Tests for acute infection with influenza, Epstein-Barr virus, cytomegalovirus, herpes simplex virus, HHV-6, West Nile virus, mycobacteria, cryptococcus and coccidioides were negative. Taken together, these studies support the diagnosis of AHLE.

Due to the clinical improvement in our prior patient, treatment with high-dose intravenous corticosteroids, hypertonic saline, IVIG, and anakinra 100 mg subcutaneously daily was initiated early in his course. Six months after the initiation of treatment, MRI revealed improvement of edema and evolving necrosis at the corpus callosum, without evidence of new white matter lesions (Figure 1d). While on anakinra therapy, the CSF myelin basic protein decreased (16.5 ng/mL) and leukocytosis resolved. His CRP and ESR have remained less than 0.5 mg/dL and 7 mm/hr, respectively. He participated in an intensive inpatient rehabilitation program leading to gradual improvement in fine and gross motor skills, as well as speech, and was discharged after 2 months, an encouraging outcome in this severe disease. Attempts to wean him from anakinra have led to relapse of symptoms, including decreased balance, speech difficulty, and increased spasticity, all of which improve with re-instating anakinra therapy. Two years after his initial presentation, he continues on anakinra 100mg administered subcutaneously daily.

As with Patient A, we identified abnormalities in the alternative complement pathway with a FI level of 6 µg/mL, and Factor H of 136 µg/mL. Consistent with these levels was C3 of <40 mg/dL, C4 of 52 mg/dL, CH50 of 40 U/mL, and AH50 that was reduced to 29% of normal. Complement studies on his immediate family members revealed mildly reduced FI levels in the mother, father and brother.

Molecular findings

As reduced FI levels were the most significant abnormality detected in our patients, we focused on the *CFI* gene. Sequencing the 13 exons of *CFI* in our two patients revealed two

novel missense mutations. Patient A and her sister are compound heterozygotes for the glycine 71 to valine (G71V) amino acid substitution encoded by exon 2 and the cysteine 196 to serine (C196S) amino acid substitution encoded by exon 4, while her mother, father and brother are heterozygotes with single *CFI* mutations, consistent with their reduced serum FI levels (Figure 2, *Left*). Although his FI serum levels are consistent with 2 affected alleles, Patient B is only heterozygous for the exon 2 mutation and lacks any other *CFI* coding or splice site mutations. His father is also heterozygous for the exon 2 mutation and no mutations were identified in his brother or mother, despite the mildly reduced FI levels similar to the heterozygotes from Family A (Figure 2, *Right*). Neither mutation was found in one hundred Filipino control alleles.

We modeled these mutations and more than 40 identified mutations in *CFI* [4, 12–16] on the FI structure [17] (Figure 3). The G71V substitution clashed with the core of the Factor I/ membrane attack complex (FIMAC) domain. The C196S substitution disrupted a disulfide bond in the scavenger receptor cysteine-rich domain (SRCR). Remarkably, both substitutions could change the packing of the helical linker, thereby disrupting the disulfide bond between the helical linker and the light chain (Figure 3, violet). Thus, these mutations may cause folding problems, and/or early clearing of FI through protein aggregation or degradation, thereby reducing the FI levels.

Previously described mutations in *CFI* have been shown to affect the secretion and/or function of FI [13]. To further evaluate the effect of these mutations on the FI protein, we utilized an *in vitro* transfection system to express wild-type and mutant FI. Intracellular FI was detected by flow cytometry similarly in the wild-type and mutant *CFI* transfected cells suggesting no effect of the mutation on the rate of protein translation (Figure 4a). However, subsequent analyses of the cell culture supernatants demonstrated that no FI protein could be detected from cells transfected with constructs containing either of our patients' mutations (Figure 4b). In contrast, cells transfected with plasmid containing wild-type *CFI* produce detectable protein that was maintained in the supernatant for at least 24 hours, consistent with previous reports of FI half-life (not shown) [18].

Complement activation is increased in the brain parenchyma of patients with AHLE

We suspect that in our patients, similar to patients with other *CFI* mutations [12, 19], uncontrolled activation via C3 occurs, leading to increased C3b production and a relative decrease in circulating C3 levels [8], consistent with our patients' laboratory studies. Immunohistochemical evaluation of brain biopsy tissue from Patient B, revealed such an increase in C3, as well as increased membrane attack complex (MAC) deposition on neuronal cell surfaces compared to normal brain tissue (Figure 5a,b, Supplemental Figures S1,S2). In addition, immunohistochemical interrogation of the brain biopsy tissue of Patient B demonstrated IL-1 β positivity (Figure 5c) that was not observed in normal brain tissue (Supplemental Figure S1), supporting the use of anakinra as a specific treatment of AHLE.

DISCUSSION

FI is a plasma phase, serine protease responsible for inactivation of C3b and C4b, thereby preventing the progression to C5 convertases in both the alternative and classical pathways (summarized in Supplemental Figure S3). FI is produced primarily by the liver, but can also be produced by monocytes, keratinocytes, and astrocytes [20]. Although there are very few patients identified with immunodeficiency due to FI deficiency, there is a growing list of disorders associated with similar uncontrolled MAC activation, including atypical Hemolytic Uremic Syndrome (aHUS) [21], macular degeneration [22], and now AHLE. Patients with complete FI deficiency typically have increased susceptibility to pyogenic infections with encapsulated bacteria. Partial FI deficiency has been associated with immune

complex-mediated diseases and aHUS (Reviewed in [12]), but a risk for demyelinating disease in these patients, has not been previously described. The clinical challenges of these cases reflect the intricate play of complement within the specialized neuromicroenvironment.

The role of complement in the neuromicroenvironment has been shown to be a double-edged sword, implicated in both cytolytic control of infection and neurogenerative recovery [23]. Although it has been shown that neuronal cells are capable of producing complement factors for control of local inflammatory reactions, they are also exquisitely sensitive to their cytotoxic effects and lysis via the MAC [7, 24]. Complement may be activated by intrinsic CNS proteins, including myelin [25, 26], and dysregulation of complement has been implicated in numerous neurologic diseases [27]. Additionally, there is evidence that complement plays a significant role in murine experimental autoimmune encephalitis (EAE), a model of acute demyelination [23, 28]. Thus, an inflammatory insult may result in amplification of complement-mediated cell death within the brain parenchyma by both a passive transfer of cytokines from the peripheral circulation, and by a secondary central increase in other complement proteins in response to these mediators. We propose that this feedback loop (Supplemental Figure S3) may be the mechanism behind AHLE in the context of partial FI deficiency.

The spectrum of medical phenotypes for FI deficiency, ranging from asymptomatic to severe, is reflected in the literature and it is interesting to note that many of the described patients have only one *CFI* mutant allele [14–16]. Her requirement for additional aggressive therapy, including anakinra, during relapse of her disease suggests that the cascading effects of complement may also divide patients into differing response groups. Earlier reports described families in which the proband with FI deficiency had recurrent infections from childhood, but their respective siblings, with similar serum complement studies, remained healthy [29, 30], reminiscent of the affected, yet asymptomatic sister of Patient A. The mechanism of this phenotypic variation is unknown and suggests that the siblings may not have been exposed to an environmental trigger or may express an additional protective immune mechanism that modulates the effect of the FI deficiency.

We identified two novel mutations in *CFI* that were not present in ethnically matched controls, and result in failure to secrete protein in an *in vitro* transfection system, similar to nearby mutations described in aHUS (C43F, FIMAC domain, and N151S, SRCR domain) [13]. Interestingly, there does not appear to be a correlation between the site of the mutation and subsequent phenotype. In our patients, the primary site of inflammation was in the CNS, whereas the structurally adjacent mutations such as P50A, R317W, and particularly P64L, which could have similar steric effects on FI protein, have only been described in aHUS [12, 14–16]. It is currently unclear why some point mutations led to AHLE and nearby mutations are associated with aHUS. Subsequent clinical laboratory studies in our patients have consistently demonstrated normal renal function, and clinically there has been no increase in frequency of infections. As both of our patients are of Filipino descent, their phenotype may be the result of these mutations on a certain genetic background. Clearly additional patients are required to resolve these questions.

The success of therapy with anakinra is suggested by the improved clinical courses for both of our patients. Interleukin-1 β (IL-1 β) is a pro-inflammatory cytokine prominent in many inflammatory diseases [31]. The activity of IL-1 β is specifically regulated by IL-1Ra, which competes with IL-1 β for receptor binding without inducing signal transduction. An imbalance between IL-1 β and native IL-1Ra has correlated significantly with disease severity in acute and chronic neuroinflammatory disorders such as brain ischemia, Alzheimer's disease, and multiple sclerosis [32–35]. Furthermore, in rat models of EAE,

administration of recombinant IL-1Ra led to reduced disease severity compared to controls [36]. The identification of IL-1 β in the brain biopsy tissue from our patient, which was not seen in normal brain tissue, further supports the use of anakinra as a specific treatment of AHLE. The similarities between the effects of IL-1Ra on slowing the development of EAE and in ameliorating the neurologic findings of AHLE in our patients, suggest that the pro-inflammatory state induced by CFI deficiency is amenable to more targeted immunomodulatory therapies.

We propose that IL-1 inhibition slows neutrophil recruitment and complement protein production [37], reduces MAC formation [38], and prevents further neuronal cell lysis (Supplemental Figure S3). We suspect that in Patient A, anakinra served to control inflammation, including complement-mediated effects by reducing IL-1 β signaling [27, 37]. Ultimately, she was weaned from therapy. Patient B, however, has an ongoing requirement for anakinra and complement studies performed while receiving therapy suggest persistent complement consumption with low C3 (31 mg/dL). Thus, despite the similar presentation and serum FI levels in our two patients, anakinra may not be sufficient for full recovery in all cases of AHLE associated with partial FI deficiency. These cases may be amenable to other complement inhibitors, but this approach requires further investigation [39].

Our report has several limitations. Due to the rarity and high mortality rate of AHLE, we are limited to our experiences with two pediatric patients who were treated at a single institution. Similar to descriptions of patients with aHUS, we have identified an association between partial FI deficiency due to mutations in *CFI* and AHLE, however an exact causal relationship cannot be proven. Although FI was detected at low levels in both patients' serum and our data indicates that the mutated FI protein is translated *in vitro* similar to wild-type FI, there was undetectable mutant FI in the transfected cell culture supernatants. Flow cytometry of transfected cells without the protein transport inhibitor Brefeldin A demonstrates that low amounts of mutant FI protein are not retained in the cytosol (data not shown). Therefore, it is not clear if degradation of the mutant protein occurs intracellularly prior to secretion or extracellularly. While Nilsson et al have successfully used this six-histidine tag to identify secreted mutant FI [6], it is possible that the combination of our patients' mutations and the tag affects secretion. It is also possible that these mutations affect function, but we were unable to perform these assays. Complement is activated in response to numerous stimuli [24, 40] and may be increased in the brain in response to ischemic-reperfusion injury, infection and traumatic lesions, as well as in AHLE. Thus, the deposition of C3 and MAC in our brain biopsy samples may not be unique to AHLE or FI deficiency, but suggests an opportunity for complement-targeted therapeutic intervention that may not have been previously appreciated in neuroinflammatory diseases.

CONCLUSIONS

We have described, for the first time, two individuals with partial FI deficiency and AHLE, a previously unreported phenotype for FI deficiency, supporting a role for complement in the pathogenesis of this disease. We propose that in these predisposed individuals, an infectious insult may trigger uncontrolled activation of complement in the brain parenchyma leading to profound neuroinflammation and demyelination. This is the first evidence of a role for IL-1 and complement in AHLE, and the success of treatment with anakinra observed in our patients may offer both an important adjunctive therapy for this disease and insight into the pathophysiology of demyelinating syndromes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AHLE	Acute Hemorrhagic Leukoencephalitis
aHUS	atypical Hemolytic Uremic Syndrome
CFI	Complement factor I gene
CNS	central nervous system
CSF	cerebrospinal fluid
EAE	experimental autoimmune encephalitis
FI	Complement Factor I
FIMAC	Factor I/membrane attack complex
IL-1β	Interleukin-1 β
IL-1Ra	IL-1 receptor antagonist
MAC	membrane attack complex
SRCR	scavenger receptor cysteine-rich

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FIG. 1. Brain imaging and biopsy

a,c, Brain MRI, Axial T2, on admission for Patient A (**a**) and Patient B (**c**). **b,d**, Brain MRI, Axial T2, Post-discharge. **e,f**, White matter with multiple foci of perivascular myelin degradation, hemorrhage, edema, and scattered infiltrating neutrophils (arrowheads), foamy macrophages and hemosiderin-laden macrophages in brain biopsies from Patient A (**e**) and Patient B (**f**). (H&E, X100)

FIG. 2. FI mutations and serum levels

Pedigrees identifying mutations in *CFI* and their respective serum complement factor I (FI) levels (normal 29–59 $\mu\text{g/mL}$). Probands with AHLE are indicated by arrowheads. *Left*, Family A. Two novel mutations identified in the *CFI* gene. Patient and her sister are compound heterozygotes. *Right*, Family B. Patient and father share the G71V mutation also found in Family A. A second coding mutation was not identified, but a noncoding or regulatory variant could also explain the markedly decreased FI serum levels

FIG. 3. Molecular modeling of CFI mutations

a, Domain structure with the positions of mutations associated with recurrent infections, aHUS, and AHLE [4–6, 12–16]. Mutations are numbered inclusive of the signal peptide (+18 relative to position numbers when the signal peptide is excluded) [13]. **b**, Structure of human FI [17] with AHLE mutations (red spheres) **c**. Structure with aHUS mutations (red spheres). Molecular images generated by PyMol [41]. *FIMAC*, Factor I/membrane attack complex; *SRCR*, scavenger receptor cysteine-rich

a

b

FIG. 4. Recombinant FI protein expression

a, Flow cytometry demonstrates similar intracellular expression of wild-type FI and mutants in transiently transfected HEK293 cells following treatment with Brefeldin A. **b**, Western blot of purified cell culture supernatants following transient transfection with wild-type (wt) or mutant *CFI*His-tagged plasmids. Images are representative of five independent experiments

FIG. 5. Histologic analysis of brain tissue

a–c, Immunohistochemistry of brain biopsy sections from Patient B demonstrating positive staining for C3 (*a*, *X100*), C5b-9 (MAC, *b*, *X400*) and IL-1 (*c*, *X100*). Arrowheads indicate positively staining cells.