Complement Factor I Deficiency Associated With Recurrent Meningitis Coinciding With Menstruation

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Background: Complement (C) factor I deficiency is a rare immunodeficiency state frequently associated with recurrent pyogenic infections in early infancy. This deficiency causes a permanent uncontrolled activation of the alternative pathway resulting in massive consumption of C3.

Patient: A 23-year-old woman with monthly recurrent meningitis episodes, mostly in the perimenstrual period, since August 1999. Previously, at age 16 years, she had meningococcal sepsis, also coinciding with menstruation.

Objectives: To study the patient and her family to elucidate the molecular defects in the pedigree and to evaluate her clinical evolution.

Results: We describe clinical, immunological, and treatment follow-up during this period. First, we characterized the existence of a total complement factor I deficiency defined by undetectable levels by enzyme immunoassay. This total deficiency was also found in her sister. Her parents and brother had approximately half of the normal levels. In addition, the patient had very low levels of C3; factor B; and an important reduction of factor H, properdin, C5, C7, and C8 complement components. Additional studies in the patient’s sera evidenced high levels of immune complexes containing C1q and immunoglobulin (Ig) G, as well as C3b/factor H, C3b/properdin, C3b/IgG, and properdin/IgG complexes. Treatment with prophylactic antibiotics, antiestrogen medication, plasma infusions, or intravenous immunoglobulin has been unsuccessful in avoiding consecutive meningitis episodes.

Conclusion: For the first time to our knowledge, these data present an unusual relationship between meningitis episodes and menstruation in factor I immunodeficiency.

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HEREDITARY deficiency of factor I is a rare autosomal recessive condition. To date, 33 homozygous individuals from 23 different pedigrees have been described. The molecular basis of the deficiency has been resolved in only 3 pedigrees. The clinical manifestations usually begin in early childhood and consist essentially of severe recurrent pyogenic infections mainly caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae, as well as an increased incidence of glomerulonephritis and systemic lupus erythematosus-like illness. Homozygous patients have low levels of complement (C) 3 and factor B, reduced levels of factor H and, to a lesser extent, of properdin (P) and the terminal complement components. Heterozygous individuals are often asymptomatic and have normal C3 and factor B values, with plasma concentrations of factor I of about 50% of the normal range.

Human complement factor I is a plasma serine proteinase that plays an essential role in the modulation of the complement cascade. Factor I cleaves the α’ chains of C4b and C3b and thereby is involved in the regulation of both the classical and alternative C pathways. Factor I function is dependent on various cofactors: the cleavage of C4b requires C4-binding protein (C4bp), and the cleavage of C3b is dependent on complement factor H. Cell-surface molecules such as complement receptor 1 (CD35) and membrane cofactor protein (CD46) act as factor I cofactors on host tissues. By its action on C3, factor I prevents formation of the alternative pathway C3 convertase and thereby regulates the amplification loop of the alternative pathway. Factor I is an 88-kd plasma glycoprotein composed of 2 disul-
PATIENTS, MATERIALS, AND METHODS

PATIENTS

Serum samples were obtained from all 5 family members, aliquoted, and immediately frozen at –80°C until use. Informed consent was obtained from the patient and the other family members.

METHODS

Complement Studies

Serum concentrations of immunoglobulins, C3, C4, and factor B were measured by nephelometry. Concentrations of C1q (The Binding Site, Birmingham, England) and C1 inhibitor (C1-INH) (Dade Behring, Marburg, Germany) were determined by radial immunodiffusion. Circulating immune complexes (ICs) containing immunoglobulin (Ig) G and C1q were measured by commercial enzyme immunosorbent assay (ELISA) (Scimedx Corp, Denville, NJ). Functional activity of the classical (CH50) and the alternative (AP50) pathway of complement was measured by hemolytic assays according to standard procedures. C3-nephritic factor (C3-NEF) activity was measured by hemolytic assay. Activities of C7 and C8 were also measured by hemolytic assays by using deficient sera from previously diagnosed patients as described. Initial screening for qualitative estimation of complement components was performed by double immunodiffusion (Ouchterlony analysis) with polyclonal antibodies against most of the C components.

Complement Components, Anti–Factor I Autoantibodies, and ICs Quantitation by ELISA

Further quantification of P, factor H, factor I, and C5 was performed by sandwich ELISA, all developed in our laboratory. Briefly, plates were coated overnight with either a purified IgG fraction from goat anti-P antibodies (1 µg per well) (ATAB; Atlantic Antibodies, Scarborough, Me), purified IgG from either anti–factor H or anti–factor I goat antiserum samples (1 µg per well and 2 µg per well, respectively) (Quidel, Mountain View, Calif), or mouse monoclonal IgG anti-C5 (40 ng per well) (Quidel). In each case, purified P, factor H, or factor I protein, or a C5 calibrated serum (Dade Behring), was included for standard calibration curves. Appropriate serum dilutions from pooled normal human serum (NHS) and from each member of the family were included. After an hour incubation, murine monoclonal anti-P (1/2 K diluted) (Chemicon, Temecula, Calif), anti–factor H (1/8 K diluted) (Quidel), anti–factor I (1/20 diluted supernatant) (Serotec, Oxford, England), or goat immunoglobulins anti-C5 (1/1 K diluted) (ATAB) was used as the detecting antibody.

Anti–factor I autoantibodies (IgG, IgM, or IgA) were also checked by ELISA by coating purified factor I (50 ng/well) (Quidel) to the plates. In each case, the reactions were visualized by the appropriate peroxidase-conjugated antibodies, using ABTS/H2O2 (2’,2’ azino-di-[3-ethylbenzthiazolesulfonate (6)] diammonium salt/hydrogen peroxide) as substrate.

To detect possible C3/factor H, C3/P, C3/IgG, and P/IgG complexes in factor I–deficient serum, ELISA was performed. Briefly, plates were coated with either monoclonal anti-C3 antibody (from A. Torano, PhD, Instituto de Salud Carlos III, Madrid, Spain) or goat purified immunoglobulins anti-P (ATAB), saturated, and then incubated with NHS or the patient’s deficient serum (1/1 K diluted). To search the molecule eventually bound to the captured antigen, several antibodies for testing the presence of each of these complexes in serum samples were added: polyclonal anti–factor H, rabbit polyclonal anti-C3 (DAKO A/S, Glostrup, Denmark) or peroxidase conjugated anti-human IgG (Nordic Immunology, Tilburg, the Netherlands). The reaction was developed with an appropriate peroxidase secondary antibody where needed.

Western Blot Test

The Western blot test was also performed to verify factor I deficiency. Briefly, serum samples (1 µL) from all family members were run on 10% SDS-PAGE (sodium dodecylsulfate-polyacrylamide gel electrophoresis) under nonreducing conditions. After running the samples, the gel was transferred to a nitrocellulose sheet and the membrane probed with an anti–factor I polyclonal antibody (1/500 diluted). The reaction was revealed with a phosphatase-labeled secondary antibody and NBT/BCIP (nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate) as precipitating substrate.

fide-linked polypeptide chains (α, 50 kd; β, 38 kd) that circulates in an active form at a concentration of 30 to 50 µg/mL. In the absence of factor I and/or factor H, there is an uncontrolled formation of C3 convertases, which results in marked production of C3b, sufficient to provoke consumptive secondary immunodeficiency of component C3. Low levels of C3 may impair immune complex metabolism and may reduce phagocytic activity, opsonization, and antibody production induced by IC3b, C3dg, and C3 cleavage fragments produced after the action of factor I.

We describe a Spanish factor I–deficient woman (age 23 years) who has a history of recurrent meningitis episodes coinciding with the perinatal period for approximately 18 months (February 2001). Her family is also described.

RESULTS

CLINICAL HISTORY AND TREATMENT OF THE PATIENT

The proband was in good health until age 11 years when she had an acute-onset abdominal pain and underwent appendectomy. At age 16 years she had meningococcal sepsis coincident with menstruation. Then she had recurrent tonsillitis and underwent tonsillectomy 1 year
Later. From age 22 years until February 2001, she has had monthly episodes of acute meningitis around the perimenstrual period (a total of 20 episodes). After the fourth consecutive meningeal episode, she was studied for a possible meningeal fistula with no conclusive results, but she underwent surgical intervention by bifrontal craniotomy to stop possible nasal cerebrospinal fluid (CSF) loss and to prevent meningitis; however, she had another meningeal episode. For 4 months the patient was treated with a daily dose of 500 mg of sodium cefuroxime as prophylactic. After the fifth episode, she was referred to our hospital as she had low C3 levels. At each episode she was treated with sodium cefotaxime, 2 g every 4 hours for a minimum of 1 week. She had good evolution while receiving this treatment and recovered from cephalgia and fever in a few days. After 8 consecutive episodes, and because of the coincidence with menstruation, she was treated with triptoreline pamoate (3.5 mg, an antiestrogen drug, the effects of which last for 3 months), but under this treatment, periodical meningitis continued. Subsequently, she was also treated with plasma infusions, 25 mL/kg of weight, every 15 days, but she continued having meningeal episodes. Since all bacterial cultures of her CSF were sterile, Mollaret meningitis or an inflammatory situation was considered, and she was treated with daily corticoids (prednisone, 1.5 mg/kg body weight) owing to allergy to nonsteroidal anti-inflammatory agents. During this treatment, positive polymerase chain reaction findings for *N meningitidis* type B DNA was found in the CSF, and the patient was treated with penicillin G benzatine (1.2 million units), and later, after a new meningeal episode, she was treated with rifampicin, 300 mg twice a day. Three months after triptoreline treatment, she had normal menstruation and meningitis periodically continued without being as strictly related with the menstrual period. To discard allergic or toxic meningitis owing to medications, provocative tests were performed with the drugs she habitually received: amoxicillin trihydrate, metamizol magnesic, and diclofenac sodium (Voltaren; Novartis Farmaceutica SA, Barcelona, Spain), all test results being negative, with the exception of diclofenac, which induced a rash reaction owing to her allergy to nonsteroidal anti-inflammatory agents, without her developing meningitis. After that, she continued under care at the hospital and was treated with intravenous γ-globulin (Endobulin; Baxter SL, Valencia, Spain) infusions at the immunomodulatory dose of 1.5 g/kg of weight every 10 days, but she has continued having meningitis except during 1 menstrual period. At present, the dose of γ-globulin has been reduced to 1 g/kg body weight every 15 days to avoid the possibility of hyperviscosity syndrome. The patient has recovered from all the episodes without any sequelae.

**ANALYSIS OF CSF FROM MENINGITIS EPISODES**

Glucose was diminished and protein levels were highly elevated, indicating an infectious situation. Leukocytes were raised to 1550 per mm³ (85% were polymorphonuclear cells and 15% monocytes). Beta-2-microglobulin was within the normal range. Bacteriological cultures of CSF were always sterile. In 2 independent samples of the CSF, DNA from *N meningitidis* group B was detected by polymerase chain reaction, but on 3 other samples, it was negative. Findings for herpes virus in CSF were also negative, and only once was cytomegalovirus positive by polymerase chain reaction.

**FAMILY HISTORY**

The patient has 1 sister and 1 brother who are 9 and 5 years younger, respectively. Both of them have no history of increased susceptibility to infections. Her parents are both healthy, and there is no history of consanguinity although they come from the same village in the south of Spain. She had another brother who died of meningitis when he was 7 years old (Figure 1).

**IMMUNOLOGICAL STUDIES**

Investigation of the patient revealed a normal complete blood cell count and basic biochemical profile (including hormones). Lymphocyte markers and function were normal. The immunoglobulin concentrations were within the normal range, except for a polyclonal increase of IgM (IgM =500 mg/dL, reference range: 40-260 mg/dL). The IgG subclass concentration gave no evidence of selective subclass deficiency. Antinuclear antibodies (measured by indirect immunofluorescence on Hep-2 cells) and rheumatoid factor (measured by latex agglutination) were not detected. Other autoantibodies such as anti-myeloperoxidase, anti-proteinase 3, anti-β2 glycoprotein I, and C3-NEF were not detected. Tetanus and pneumococcal antibody titers were within the normal range.

Her complement system was investigated together with that of all 4 family members (Table). The results demonstrated the diagnosis of a total factor I deficiency in the patient and in her asymptomatic younger sister, since factor I was undetectable by Ouchterlony analysis. This absence was confirmed by ELISA in both siblings as there was no detectable AP50 and CH50, and both had reduced levels of C3, C5, factor B, P, and factor H proteins. Activities of C7 and C8 were also reduced in both siblings. Immune complexes were also elevated in both, although remarkably higher in the patient. Both parents and her brother had approximately half the normal concentration of circulating factor I, together with a slight decrease in AP50 values. All these data indicated the

![Figure 1. Pedigree, age, and clinical manifestations of the family members and patient (arrow). Father (I-1), aged 48 years, healthy. Mother (I-2), aged 42 years, healthy. Daughter 1 (II-1), aged 23 years, propositus. Son 1 (II-2), died (aged 7 years) from meningitis. Son 2 (II-3), aged 17 years, asthma. Daughter 2 (II-4), aged 13 years, healthy.](image-url)
Inheritance of the trait throughout the family in an autosomal recessive manner (Figure 1). Western blot studies are concordant with the diagnosis and showed no other factor I anomalous species (Figure 2).

**COMPLEMENT AND IMMUNE COMPLEXES ANALYSIS**

The different clinical treatments induced some changes; during plasma infusions, instead of the lack of successful results for preventing meningitis, C3 plasma values were raised nearly to normal levels, and AP50 and CH50 were normalized. In addition, circulating immune complexes were lowered with these infusions and more so when the patient received corticoids and rifampicin (data not shown). Intravenous immunoglobulin also produced a great reduction of these immune complexes (to \( \approx 30 \mu g/mL \), reference value, \( \approx 35 \mu g/mL \)). The ELISA experiments evidenced the presence of C3b/factor H, C3b/P, and P/IgG complexes (Figure 3). Experimental conditions showed that these complexes were present throughout the study. These also were found in other patients with different diseases and the presence of immune complexes, but they were always negative in the tested controls (n=10) and in a serum pool from 30 normal controls.

**COMMENT**

This is the first description to our knowledge of a factor I–deficient family in Spain, with 23 other pedigrees previously being described.\(^1\)–\(^4\) This pedigree presents 1 healthy homozygous sibling, while to our knowledge, only 2 healthy homozygous siblings have been previously described.\(^15\) Moreover, the homozygous patient presents a very unusual and dangerous situation owing to the extraordinary frequency of the meningeal attacks. Factor I deficiency causes severe secondary C3 and factor B depletion; in addition, the C profile in this patient showed a

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<th>Variable†</th>
<th>I 1</th>
<th>I 2</th>
<th>II 1</th>
<th>II 3</th>
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<td>&gt;100‡</td>
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<td>45‡</td>
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*CH50 indicates total hemolytic complement; C3-C8, complement C3 to complement C8; C1-INH, complement C1 inhibitor; AP50, alternative complement pathway; NHS, normal human serum; ellipses, undetectable; P, properdin; and ICs, immune complexes.
†Normal values of factor I, P, C7, and C8 were determined with a pool of NHS (n = 30).
‡Altered values.

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Figure 2. Factor I antigen detection by Western blot test on the patients’ serum samples. All 5 family members’ serum samples were included in consecutive lanes. Mks indicates molecular weight markers; NHS, normal human serum. The arrow indicates purified factor I migration.

Figure 3. Levels of complement C3/factor H, C3/properdin (P), C3/immunoglobulin (Ig)G, and P/IgG complexes in patient’s (II 1) and normal human serum (NHS). The mean±SD optical density is plotted; n=5.
reduction in many other C components such as P, factor H, C5, C7, and C8. This situation was coincident with other factor I–deficient patients described earlier.1 Apparently, this patient has all the C components altered with the exception of the initial classical C components. This profile is compatible with the excess activation on the fluid phase of the alternative C pathway. Furthermore, factor I deficiency interferes with the formation of C3 fragments, which are required for efficient phagocytosis and B-cell memory (iC3b and C3dg, respectively). The lack of both types of fragments may also explain the predisposition for pyogenic infections.4,5

The patient also presents highly elevated levels of ICs, without any consumption of the classical C components (C1q or C4). This feature is uncommon, although the presence of ICs has also been seen in other factor I–deficient patients.10,16,17 In our case, the presence of C3b/factor H complexes could also be causing the negative regulation of C3 convertase and producing an increase in C consumption. Binding of C3b to factor H might act as a mechanism for the inactivation of some of the biological effects of excess fluid-phase C3b. Factor H- and C3-altered mobility in agarose electrophoresis gels has been reported in other factor I–deficient patients.9,10 Factor H in the proband’s serum migrates toward the anode with higher mobility than factor H from the normal control (data not shown). This abnormal migration could possibly be owing to the formation of these C3b/factor H complexes, since in the absence of factor I, C3b persists in high levels and may increase its binding to factor H. Furthermore, taking into account the predicted existence of C3b/IgG complexes in normal serum,19,20 we have shown the presence of these complexes by ELISA experiments. Results show that C3/P, P/IgG, or C3b/IgG complexes are present in the serum of this patient at a higher concentration than in NHS. Moreover, these C components could be involved in a large molecular complex (C3b/P/IgG) as predicted by Lutz et al.19 and in agreement with the proposed mechanism, complexes could preserve this excess of C3b as described by Jelezarova and Lutz.20

The main causes of recurrent meningitis were taken into account, and most of them were discarded. A para-meningeal focus could never be evidenced; moreover, cryptococcal cultures were always sterile as were Borrelia cultures, and the patient never had Behcet disease. The clinical situation was not like familial Mediterranean fever since her episodes were benign and clinical symptoms such as cephalgia and fever remitted in 2 to 3 days. The cause of these meningitides (infectious or Mollaret) is unclear. The possibility that they were all infectious is scarcely supported since standard microbiological cultures for CSF were always sterile. Moreover, polymerase chain reaction was positive twice for N meningitidis type B DNA and negative 2 more times in 4 different CSF samples. Furthermore, we could not rule out that some of these were septic and others aseptic as both types were possible. At each episode, antibiotics were administered, and the patient’s clinical symptoms diminished with this treatment. We did not obtain results without antibiotic treatment since the risk of worsening the condition was too great. Moreover, the patient and family always rejected this option. Molar-et recurrent meningitis was also found in another factor I–deficient woman.21

Cerebral endometriosis has been described as being associated with intermittent focal headaches,22 causing catamenial epilepsy23 and subarachnoid hemorrhage,24 but in this patient, meningeal hemorrhage has never been found as the absence of red cells in the CSF has proven. Moreover, meningeal episodes have continued in the absence of menstruation as happened with triptoreline treatment. The eventual relationship of meningitis with menstruation is unknown. In a different situation, as hereditary angioedema caused by C1-INH deficiency, hormonal factors can regulate the episodes of this disease; for example, menstruation could be a triggering factor for episodes, and pregnancy is also a condition that can improve or worsen the course of this disease. Furthermore, androgens such as danazol are used for treating this angioedema.25,26 Taking this into account, we can hypothesize that an unknown factor or mechanism related to hormonal regulation in this complement deficiency, and meningitis could be an underlying factor. Some authors have shown that several components of the complement system exist in the human endometrium in a hormone-dependent manner and may play a role in the normal reproductive function.27 Recently, Nowicki et al28 have shown that there is a higher susceptibility to gonococcal infection by Neisseria gonorrhoeae during the menstrual cycle, and changes in antibacterial activity are correlated with serum complement activity. These authors suggest that similar cyclic changes could be involved in the pathogenesis of other infectious diseases in women. Treatment with triptoreline has not abolished the meningeal episodes in this case, but other hormonal factors could be involved.

In conclusion, we describe a factor I–deficient patient with an unusually high number of meningeal episodes with resistance to several treatments, including plasma infusion, anti-inflammatory drugs, and prophylaxis with antibiotics, the latter being previously suggested in this1 and other C deficiencies.29 The molecular basis of this deficiency is being studied to characterize this patient’s genetic defect.

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