Neonatal Purpura Fulminans: A Genetic Disorder Related to the Absence of Protein C in Blood

By Ewa Marciniak, H. David Wilson, and Richard A. Marlar

To confirm the pathogenesis and the genetic background of neonatal-onset purpura fulminans, two unrelated infants with this rare thrombotic syndrome and 47 of their asymptomatic relatives were studied. In both families, 27 subjects with hereditary partial deficiency of protein C, including both parents of each patient, were identified. The patient in whom it was possible to evaluate protein C directly showed no detectable levels of this plasma component. These findings confirm the linkage of neonatal purpura fulminans to a genetic trait with established mendelian transmission and strongly suggest that the syndrome is an expression of homozygosity for protein C deficiency.

The dramatic clinical picture and the type of pathologic change that develops as a result of the lack of circulating protein C emphasize the vital role of this protein in protection from thrombin generation, mainly within the microvascular system. However, our data do not contribute to the evidence that partial familial protein C deficiency is associated with a major risk of venous thromboembolism.

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PURPURA FULMINANS, first described in the late 19th century,1 is a rare, frequently fatal syndrome occurring predominantly in children. It is characterized by massive, progressive bleeding into the skin, accompanied by widespread thrombosis of capillaries and venules, with subsequent necrosis of affected tissues. Occasionally, larger vessels and organs other than skin are involved. The hematological picture corresponds with disseminated intravascular coagulation. It is well recognized that in this disorder, thrombosis is the primary event directly responsible for both the hemostatic defect and bleeding,2 but the nature of the pathogenic factor leading to thrombotic changes has not been established. In the acquired syndrome, which usually appears following a benign infection, some immune mechanism is generally suspected.3 However, familial incidence in the neonatal form of purpura fulminans, described by van der Horst,4 and its association with hereditary protein C deficiency56 suggest a genetic background.

We have diagnosed purpura fulminans in two unrelated newborn infants in whom we identified homozygosity for familial protein C deficiency as the cause of the disease syndrome. Because plasma protein C represents the precursor of a proteolytic enzyme with potent anticoagulant properties,79 this finding provides a unique insight into the physiology and pathology of blood coagulation and helps to establish the critical role of protein C in modulating intravascular events. A remarkable family history of one of the probands strengthens the evidence that neonatal purpura fulminans is a hereditary disorder. Clinical and laboratory data in numerous family members heterozygous for protein C deficiency are presented to complement the recently published information on the association of isolated partial protein C deficiency with the risk of thrombosis.10,11

CASE HISTORIES

Patient 1

The proband was a white male infant born at term after an uncomplicated pregnancy to a 17-year-old mother (gravida I, para
on the scalp, abdomen, and upper and lower extremities. On the eighth hospital day, focal seizures occurred and the child was treated with phenobarbital. An electroencephalogram, which was initially normal, demonstrated suppression of activity in the left hemisphere, predominantly in the occipital area. Subsequently, a CT scan of the head revealed bilateral, asymmetrical areas of infarction that were suggestive of superior sagittal sinus and cortical vein thrombosis. The frequency of seizures increased along with progressive general deterioration. Death occurred at the age of 32 days. Permission for autopsy was denied.

The proband was a member of a large, inbred kindred from the Appalachian area of eastern Kentucky. His parents were second cousins once removed. There has been a notion in the family about the presence of a hereditary disease that might cause skin discoloration and death of their newborn infants. The paternal grandfather of the proband (IV-10 in Fig 1), 60 years old, had four siblings (IV-3, IV-6, IV-7, and IV-13) who died at the ages of 10, 4, 5, and 4 days, respectively, after developing extensive areas of black skin changes. All other siblings survived through infancy. Their parents were said to be interrelated, but the degree of kinship is unknown. No apparent history of venous thrombosis could be established among numerous family members, except for the proband's maternal grandmother (IV-21), age 46, and another relative of the same generation (IV-4), a 67-year-old woman, both of whom suffered repeated varicophlebitis during pregnancies.

**Patient 2**

The patient was a white infant girl born at term to a 21-year-old healthy mother (gravida II, para I). At the age of 12 hours, a purpuric lesion on the right foot was noted. Similar changes, accompanied by swelling, developed subsequently on the left foot causing the admission to a local hospital, where therapy with antibiotics and heparin was initiated. The child was transferred to the University of Kentucky Medical Center at the age of 8 days (February 1969), with the diagnosis of purpura fulminans. Initial physical examination revealed a well-developed and well-nourished child with an area of necrotic skin on the left foot and a bluish-black swollen right foot with the loss of dorsal pedal pulses. There were extensive ecchymoses on the dorsum of the right hand and in the parietal region of the scalp. Bilateral cataracts were noted. On admission, the hematocrit was 24%; the prothrombin time was 14.8 seconds, with a control of 13 seconds; and the fibrinogen 85 mg/dL. Fibrin split products were markedly elevated. The platelet count on admission was 87 x 10⁹/L and decreased within the next few days to 17 x 10⁹/L. A skin biopsy performed on the eighth hospital day disclosed necrosis of the dermis with small blood vessel thrombosis and areas of hemorrhage consistent with purpura fulminans. Despite treatment with intravenous injections of heparin, antibiotics, and transfusions of blood and high molecular weight dextran, new ecchymotic lesions appeared over the face, neck, abdomen, and thighs. Terminally, the infant developed seizures and died at the age of 25 days with signs of extensive central nervous system damage.

At autopsy, extensive cutaneous and subcutaneous ischemic necrosis with widespread thrombosis of the small vessels was confirmed. Both common iliac arteries, just distal to the bifurcation, were completely occluded by thrombi. No thrombi were seen in any of the major veins, but numerous small foci of hemorrhagic infarction were found in the lungs. There was total infarction of the right kidney of about three weeks' duration. The left kidney revealed fibrin deposits in numerous small vessels and glomerular capillaries. A large amount of blood was found in the subdural space covering the cerebral hemispheres. Sections of the brain showed extensive intracortical liquefactive necrosis, with hemosiderin deposits around necrotic areas. The right eye was dissected and showed microphthalmia with an anteriorly displaced spherical lens and a fibrovascular stalk extending from the lens to the optic nerve. The retina was completely detached, and the posterior chamber contained old and recent hemorrhage.

The pedigree of the family was examined retrospectively after 15 years and is shown in Fig 2. Both of the proband's parents were healthy. They came from the same small town of eastern Kentucky where their families have resided for more than a century. There was no evidence of consanguinity in at least four generations, and no other family members were known to have had purpura fulminans. At the time of examination, the mother was in the seventh month of her ninth pregnancy. The first pregnancy culminated in the term birth of a dead infant. The proband was her first viable child. Subsequently, she had had three spontaneous abortions at the early stages of pregnancy and a pregnancy that ended in apparent intrauterine fetal death after a 30-week gestation. The product of this conception was not examined. She had two normal, well-developed children, ages 7 and 2 years. All of her seven siblings were...
alive and healthy. The father of the proband was one of 16 offspring. One sibling had died of meningitis at the age of 3 years. Four adult brothers were killed in a car accident. The paternal grandmother and both maternal grandparents of the proband were in good health. The paternal grandfather died of pulmonary emphysema at the age of 59. There was no evidence that any of the family members had venous thrombosis.

MATERIALS AND METHODS

Human protein C was purified from commercial factor IX concentrates using ion-exchange and affinity chromatography and preparative electrophoresis. Monospecific antiserum to purified human protein C was produced in New Zealand white rabbits. A Laurell-type electroimmunoassay with radiolabeled antibody was used to determine human protein C antigen in the various plasma samples according to previously published methods. The concentration of protein C in the pool of human plasma obtained from 50 healthy donors was arbitrarily defined as 100%, and all results were compared to this pool. The sensitivity limit of the assay was 3.1% because the standard pool in 1:32 dilution still gave a measurable immunoprecipitate. Protein C concentration established for normal adult population ranged from 70% to 130%. The mean concentration in 22 healthy infants was 27%, ranging from 18% to 46% (R.A. Marlar, Blood Center of Southeastern Wisconsin, and W. Hathaway, unpublished data, 1984). Children 6 months of age, or over, showed normal protein C levels.

Immunoassays for factors II and VIII, antithrombin III, and all coagulation assays were performed according to previously published procedures. The mean concentration of factor II antigen in plasma of normal subjects was 104 ± 14% and that of protein C antigen to factor II antigen was between 0.75 and 1.25. Diagnosis of isolated protein C deficiency was based on two criteria: the concentration of protein C antigen below the normal range, and the ratio of protein C to factor II below that found in the normal population. Only subjects who met both criteria were defined as having selective protein C deficiency.

RESULTS

The immunoassay performed retrospectively in the plasma of patient 1, collected on the second hospital day (third day of life) and before treatment with heparin and blood components was initiated, revealed a lack of detectable protein C (Table 1). Concentrations and activities of other vitamin K-dependent plasma proteins and antithrombin III were within the expected range for a newborn. Other coagulation factors were also present in sufficient quantities, except for notably decreased levels of fibrinogen and factors V and VIII. To establish the presence of a hereditary abnormality, 41 members of the family of patient 1 were studied. As presented in Fig 3, 23 subjects, including both parents of the propositus, had protein C concentrations below 60% of the normal level (mother's protein C was 47% and that of the father, 37%). All of these subjects had normal prothrombin levels and their protein C-factor II ratio was below the range established for a normal population. These subjects were defined as heterozygotes. They belonged to four consecutive generations. A male-to-male transmission was apparent in direct paternal line (Fig 1, subject IV-10 to V-9 and V-14, and V-14 to the propositus). In 18 investigated family members, the criteria for isolated deficiency of protein C were not met. This number includes subject V-21, who had a protein C level of 71% while her mother and three of her five children were heterozygotes. Because her husband was not available for study, a possibility of paternal transmission of the deficiency to the children was not excluded. The median age in both the protein C-deficient and the normal group was similar, as were

Table 1. Laboratory Values in Patient 1 Plasma Collected on the Third Day of His Life

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patient 1</th>
<th>Healthy Infant*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein C antigen (%)</td>
<td>Undetectable</td>
<td>27 (18–46)</td>
</tr>
<tr>
<td>Antithrombin III activity (%)</td>
<td>41</td>
<td>60 ± 16</td>
</tr>
<tr>
<td>Prothrombin AGN (%)</td>
<td>22</td>
<td>?</td>
</tr>
<tr>
<td>Prothrombin activity (%)</td>
<td>27</td>
<td>54 ± 15</td>
</tr>
<tr>
<td>Factor VII activity (%)</td>
<td>65</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>Factor IX activity (%)</td>
<td>48</td>
<td>28 (15–42)</td>
</tr>
<tr>
<td>Factor X activity (%)</td>
<td>34</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>56</td>
<td>215 ± 35</td>
</tr>
<tr>
<td>Factor V activity (%)</td>
<td>29</td>
<td>99 (40–150)</td>
</tr>
<tr>
<td>Factor VIII:C (%)</td>
<td>26</td>
<td>97 (50–180)</td>
</tr>
<tr>
<td>Factor VIII:AGN (%)</td>
<td>58</td>
<td>?</td>
</tr>
<tr>
<td>Factor VIII:vWF (%)</td>
<td>25</td>
<td>?</td>
</tr>
<tr>
<td>Factor XI activity (%)</td>
<td>10</td>
<td>?</td>
</tr>
<tr>
<td>Factor XII activity (%)</td>
<td>12</td>
<td>47 ± 18</td>
</tr>
<tr>
<td>Prothrombin time (s)</td>
<td>13.9</td>
<td>14.5 (13–17)</td>
</tr>
<tr>
<td>Partial thromboplastin time (s)</td>
<td>52</td>
<td>?</td>
</tr>
</tbody>
</table>

*Data taken from Hathaway and Bonnar, except for protein C values, which are according to R.A. Marlar and W. Hathaway (unpublished data).
the mean concentrations of prothrombin and antithrombin III (Table 2). All investigated subjects had normal prothrombin times and partial thromboplastin times.

In patient 2, who died in 1969, protein C was not evaluated. Recently, four first-degree and two second-degree relatives were studied (Fig 2). Both parents and both living siblings fulfilled the criteria for isolated protein C deficiency. In the mother’s and father’s plasma, this protein was 50% and 36%, respectively, and the brothers had 36% and 31% of protein C. The paternal grandmother and a maternal aunt had normal protein C levels. All studied relatives had normal concentrations of antithrombin III.

**DISCUSSION**

Protein C is a vitamin K-dependent plasma protein synthesized in the liver. When cleaved by thrombin, it gives rise to a potent anticoagulant enzyme that selectively destroys the coagulant activities of factors V and VIII (for review, see Seegers and Esmon). An important step toward a better understanding of the potential impact of protein C on intravascular coagulation was taken when Owen, Esmon, and Esmon showed that thrombin, bound to an endothelial cell surface cofactor, termed thrombomodulin, appears to activate protein C at an accelerated rate. It was therefore assumed that the biologically active inhibitor should be produced mainly in the microvasculature. Clinical observations indicated that patients with disseminated intravascular coagulation or adult respiratory distress syndrome, characterized by extensive endothelial cell damage, often show decreased levels of protein C in plasma secondary to increased utilization of this protein.

We have now described two unrelated infants who developed clinical, histologic, and hematologic signs of purpura fulminans shortly after birth without evidence of the extrinsic pathogens known to trigger intravascular coagulation. Each child was an offspring of two heterozygous parents with partial protein C deficiency. Patient 1 had no detectable protein C in blood; in patient 2, this plasma component was not investigated. Although protein C was not measured prior to the appearance of laboratory signs of consumption coagulopathy, the heterozygosity of the parents, the virtual absence of protein C in the initial stages of the illness, and the lack of any other known causes of purpura fulminans strongly suggest that both infants were homozygous for plasma protein C deficiency. The lack of protein C in blood resulting from this genetic condition was apparently the primary cause of the neonatal purpura syndrome. The alternative that a coagulopathy of unknown nature and origin could have resulted in secondary, fulminant depletion of preexisting low protein C levels in a heterozygote or an infant with immature hepatic synthesis appears to be highly unlikely.

A remarkable history of purpura fulminans causing death of four newborn infants was reported in the sibship of the paternal grandfather of patient 1. There were several consanguineous marriages in the family, and these infants probably received alleles from both their parents for familial protein C deficiency. Four of their five investigated siblings were heterozygotes. All of them, together with the remaining 22 heterozygotes identified in both families, were asymptomatic with respect to purpura fulminans. These data strengthen the evidence that the neonatal syndrome develops as a result of hereditary protein C deficiency and has an autosomal recessive mode of inheritance.

Three reports of neonatal-onset purpura fulminans have been published previously. The report by Van der Horst, which appeared before the existence of protein C was known, presented evidence of familial occurrence of the syndrome among siblings. Branson et al and Sills et al described purpura fulminans in two infants with protein C levels below the measurable range who were born to families with established protein C deficiency. Homozygosity in these infants was not documented, although it was not excluded. In fact, Sills et al considered homozygosity to be the case in their patient, while Branson et al thought that a combination of coumadin therapy and consumptive coagulopathy triggered by pregnancy complications led to an extreme depression of protein C levels in a heterozygote.

Congenital lack of protein C could be an exemplary model of a primary major failure in the defense mechanism. The early onset of thrombotic lesions in our patients, consistency of their appearance in the absence of extrinsic pathogens, together with the fatal course of the disease highlight the fact that exquisite control of thrombin formation within the vascular system exists under normal conditions. Microvascular
PURPURA FULMINANS AND PROTEIN C

fibrin deposits, which are the hallmark of pathology in purpura fulminans, strongly suggest that small vessels and capillaries are the main area where the regulatory function of protein C is most needed. If improperly guarded, thrombin will continuously enter the circulation and may eventually contribute to thrombosis in major vessels with organ infarction, as seen at autopsy examination in patient 2. Not without significance for the pathogenesis of purpura fulminans is the fact that activated protein C heightens fibrinolysis. Thus, the lack of sufficient fibrinolytic activity might contribute to progressive ischemia and necrosis of the tissue that is typical of this syndrome. There is no firm evidence that a thrombotic process in a homozygote with protein C deficiency originates in utero. Congenital eye changes found in both of our patients were most likely prenatal in origin, but their thrombotic nature has not been established. This raises the question of whether or not protein C or its active derivative may cross the placenta, or whether some other regulatory mechanisms to protect against thrombosis may exist in the fetal circulation.

In earlier reports on hereditary protein C deficiency, major thromboembolic complications in heterozygotes were assumed to be a consistent feature of this familial trait. Broekmans et al identified 18 subjects with isolated partial protein C deficiency in three unrelated families. In nine subjects, clinical evidence of deep vein thrombosis or pulmonary embolism was obtained, although it was not confirmed by venography or pulmonary arteriography. The authors admitted that in all families, superficial thrombophlebitis dominated the clinical symptoms. In the family described by Griffin et al, all three members with reduced levels of protein C had extensive histories of thromboembolic episodes, pointing to an abnormality similar to that seen in hereditary deficiency of antithrombin III, except that no deaths had been recorded as a result of these complications. The results of our study of a relatively large number of subjects heterozygous for familial protein C deficiency do not support the evidence that this genetic trait is associated with an increased risk of thromboembolism. Both kindreds came to our attention solely because of the occurrence of purpura fulminans in one of their infants. Only two of 27 subjects with documented protein C deficiency gave histories consistent with superficial thrombophlebitis, although nine of them were over the age of 45 years. Unlike families with congenital antithrombin III deficiency, no awareness of a familial risk of thrombosis existed in these two large kindreds. Thus, contrary to previous reports, we have not detected a thrombotic tendency in subjects with partial deficiency of protein C. The reason for this discrepancy remains unknown.

NOTES ADDED IN PROOF
Two infant homozygotes for protein C deficiency who died of massive thrombosis have been recently reported.

The mother of patient 2 recently gave birth to a healthy female child. Protein C level, evaluated in the infant immediately after birth, was 28% of the normal concentration.

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