Characterization of Multiple Quinine-Dependent Antibodies in a Patient With Episodic Hemolytic Uremic Syndrome and Immune Agranulocytosis

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A 23-year-old woman experienced six distinct episodes of severe combined neutropenia and thrombocytopenia. At least one of the episodes was accompanied by hemodialysis-requiring acute renal failure and fragmentation hemolysis (hemolytic uremic syndrome [HUS]). In retrospect, all episodes were probably associated with the ingestion of quinine. Quinine-dependent antibodies to platelets, neutrophils, T lymphocytes, and red blood cells (RBCs) were detected in the patient’s serum. By a monoclonal antibody antigen capture assay, the patient’s serum contained IgG antibodies, which in the presence, but not absence, of quinine reacted with platelet glycoprotein (GP) complexes Ib/IX and Iib/IIa, but not la/IIa. By immunoprecipitation assay, the serum, after addition of quinine, reacted strongly with an 85-Kd neutrophil membrane protein and weakly with 130- and 80-Kd moieties. Serum adsorbed with RBCs in the presence of quinine continued to react with platelets and neutrophils, and serum that was absorbed with platelets continued to react with neutrophils and RBCs, indicating that the antigenic targets were different on platelets, neutrophils, and RBCs. Since platelets and endothelial cells share some antigens, we tested patient serum for antibodies to human umbilical vein endothelial cells (HUVEC); no quinine-dependent antibodies to HUVEC were detected. However, her quinine-dependent antibodies not only bound to platelets and neutrophils, but also activated neutrophils. Thus, the patient’s serum with quinine aggregated neutrophils, but neither agent alone caused activation. Moreover, the patient’s serum with quinine (but not without) augmented the adherence of neutrophils to HUVEC. Treatment of the patient’s serum with staphylococcal protein A removed the quinine neutrophil aggregation cofactor, suggesting it was due to IgG. In both neutrophil aggregation and adherence assays, decomplementation of the patient’s serum markedly blunted its effect. Furthermore, the patient’s serum failed to aggregate formalin-inactivated neutrophils, suggesting neutrophil activation, probably by activated complement, was necessary for aggregation and adhesivity to endothelium. We conclude that our patient’s neutropenia, thrombocytopenia, lymphopenia, and anemia were due to quinine-dependent antibodies, and that these antibodies recognized epitopes that were different in the three target cell populations. We further suggest that HUS was likely secondary to the activation and adhesion of neutrophils to endothelium.

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QUININE can induce drug-dependent antibodies to platelets, and red blood cells (RBCs). 2 Idiosyncratic quinine-induced neutropenia has also been reported in some reviews, but not others. 3 Clinical manifestations of hypersensitivity to quinine include purpura, hemolysis, and acute renal failure. 4 Recently, several patients with quinine-dependent platelet antibodies have been reported to develop a full-blown hemolytic uremic syndrome (HUS) or disseminated intravascular coagulation.5,6 However, the mechanistic role of quinine-dependent antibody in these disorders has not been rigorously ascertained. We report a patient with clustered episodes of HUS and severe agranulocytosis who was taking quinine for leg cramps. We have partially characterized multiple quinine-dependent antibodies against different epitopes on platelets, RBCs, and neutrophils. In the latter instance, we found her quinine-dependent antibodies to neutrophils markedly promoted their complement-dependent aggregation and adhesion to cultured vascular endothelial cells. We suspect this interaction, if extant in vivo, might explain the HUS in our patient, as well as in those recently reported by others. These studies have been reported in preliminary form elsewhere. 10

CASE REPORT

A 23-year-old woman with a 17-year history of insulin-dependent diabetes mellitus suffered over 12 months five episodes of sudden abdominal pain, nausea, vomiting, and diarrhea associated with severe neutropenia and mild thrombocytopenia. During two of these episodes, marked fragmentation of RBCs was observed on blood smear; on one occasion, acute renal failure with an increase in creatinine to 12.0 mg/dL necessitated hemodialyses for 3 weeks and prompted the diagnosis of HUS. She had taken quinine for leg cramps, and it was recognized in retrospect that these episodes were all associated with quinine ingestion. The patient’s serum was unreactive when tested for antibodies to neutrophils in granulocyte agglutination (GA) and immunofluorescence (GIF) assays. However, in the presence of added quinine, her serum reacted strongly in both assays and did so with neutrophils from all donors tested (n = 16). The patient’s serum, again only with added quinine, also reacted with platelets in an immunofluorescence assay. Analogously, the patient’s serum alone did not react with type O RBCs; however, when quinine was added, prompt agglutination occurred. The patient was informed of these results and advised not to use quinine.

She had no further episodes of neutropenia or thrombocytopenia for 5 months, when she again developed nausea, vomiting, diarrhea, anemia, neutropenia, and thrombocytopenia (Fig 1). A peripheral blood smear demonstrated marked RBC fragmentation.
and nucleated RBCs. Creatinine on admission was 1.2 mg/dL, but rapidly increased to 2.6 mg/dL. Prothrombin time (PT) was 14.3 seconds (normal range, 11.0 to 13.5 seconds), partial thromboplastin time (PTT) 37.0 seconds (23.0 to 34.0 seconds), and thrombin time (TT) 19.2 seconds (13.0 to 18.0 seconds). By the second hospital day, the PT, PTT, and TT had returned to normal, but fibrin degradation products were 319 mg/dL (normal, <10 mg/dL). The patient denied ingestion of quinine, but quinine was detected in a February 6 urine collection. Serum samples obtained on the day of admission reacted strongly with RBCs, platelets, and neutrophils, even when no quinine was added. Serum collected on the last hospital day, 10 days later, did not react with RBCs or platelets unless quinine was added. Serum alone collected at discharge reacted weakly with neutrophils, but when quinine was added the serum reacted strongly. By immunofluorescent assay, neutrophils collected on the day of discharge, 2 days after normalization of absolute neutrophil count (ANC) (Fig 1), were strongly positive for IgG. Therefore, despite the negative drug history, we accepted this as her sixth episode of quinine-induced leukopenia/thrombocytopenia. The patient has remained well off quinine for the past 6 months.

**MATERIALS AND METHODS**

**Preparation of neutrophils.** Neutrophils were isolated by our modification of the method of Boyum from peripheral blood of normal volunteers.11,12

**Testing for drug-dependent antibodies to neutrophils.** Serum was mixed with an equal volume of quinine-HCl (Sigma Chemical, St Louis, MO) (200 µg/mL) in phosphate-buffered saline (PBS) and was tested in a GA or G1F assay as previously described.13,14 Testing for quinine-dependent antibodies to neutrophils by flow cytometry was performed in the same manner as G1F testing with slight modifications. Neutrophils were incubated in 1% paraformaldehyde (PFA) (Sigma) in PBS for 5 minutes at 20°C, washed twice with PBS containing 0.2% bovine serum albumin (BSA; Gamma Biologicals, Houston, TX), and suspended at a concentration of 10⁶ cells/mL in PBS-EDTA-BSA. Either serum or serum diluted 1:5 in PBS was mixed with an equal volume of quinine-HCl (200 µg/mL in PBS) or PBS. Serum plus PBS (80 µL) or serum plus quinine-HCl (80 µL) was incubated with 80 µL of neutrophils for 30 minutes at 37°C; the cells were then washed twice with PBS-BSA, reacted with 80 µL of fluorescence-conjugated goat antiserum to human immunoglobulin (FITC-AHG; Kallestad Diagnostics, Austin, TX), and diluted 1:180 in PBS for 30 minutes at 20°C in the dark. After being washed twice with PBS-BSA and resuspended in 80 µL PBS-BSA, the neutrophils were analyzed by flow cytometry using a FACSTAR Plus (Becton Dickinson, Immunocytochemistry Systems, Mountain View, CA).

**Testing for drug-dependent antibodies to lymphocytes.** Quinine-dependent antibodies to T lymphocytes were assayed by flow cytometry. T cells were isolated from heparinized blood using nylon wool.15 The T lymphocytes were fixed in 1% PFA and suspended at 12 × 10⁶ cells/mL in PBS-BSA, reacted with serum with and without quinine, and analyzed by flow cytometry as described above. B lymphocytes were isolated from heparinized blood using monoclonal antibodies and magnetic beads (Dynabeads HLA cell prep II, Dynal, Great Neck, NY), and quinine-dependent antibodies were assayed using a B cell cytotoxicity assay.13

**Platelet immunofluorescence.** Platelet immunofluorescence (PIF) was performed using a modification of the method of von dem Borne et al.16 Whole blood anticoagulated with EDTA was centrifuged at 300 × g for 10 minutes at 20°C. The resulting platelet-rich plasma was centrifuged at 1,300 × g for 12 minutes, and the pelleted platelets were resuspended in PBS containing 8 mmol/L EDTA (Sigma) (PBS-EDTA). The pellets were washed twice with PBS-EDTA, then fixed for 5 minutes at 20°C with 1% PFA in PBS, washed twice more with PBS-EDTA, and finally resuspended at a concentration of 2 × 10⁶ cells/mL in PBS-EDTA containing 0.2% BSA (PBS-EDTA-BSA). Serum (20 µL) or serum (10 µL) mixed with quinine-HCl (10 µL at 1 mg/mL in PBS) were incubated with 20 µL of the fixed platelet suspension for 30 minutes at 37°C. The platelets were then washed three times with PBS-EDTA-BSA, incubated with FITC-AHG in the dark for 30 minutes at 20°C, and finally washed three times with PBS-EDTA-BSA. The platelets were resuspended in 10 µL of glycercol (EM Science, Gibbstown, NJ) plus PBS (3:1 vol/vol), and analyzed under a fluorescent microscope (Leitz, Wetzlar, Germany).

**Monoclonal antibody-specific immobilization of platelet antigens assay.** Antibodies reactive with platelet membrane glycoprotein (GP) complexes IIb/IIIa, Ib/IX, and Ia/IIa were characterized by the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay as previously described,17 with our minor modifications.16

**Immunoprecipitation.** Following surface-labeling with [125I] using lactoperoxidase, neutrophils were solubilized and immunoprecipitated by our previously described method.19 For detection of quinine-dependent antibodies, serum (20 µL) plus quinine-HCl (20 µL at 200 µg/mL in PBS) was added to the solubilized [125I] surface-labeled neutrophils (100 µL), and immunoprecipitated GPs were analyzed following sodium dodeyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10% gel by autoradiography.19

**Neutrophil aggregation.** Neutrophil aggregation was performed with a dual-channel aggeregometer-recorder system (model 300-BD, Payton Associated, Buffalo, NY) as previously described.12 In brief, all assays were performed at 37°C, with cells stirring at 900 rpm in siliconized aggregometer cuvettes. Neutrophil suspensions containing 10⁷ cells/mL and 7 × 10⁶ cells/mL were used to calibrate minimal and maximal light transmission. Murine monoclonal antibody Mo 1-17 (IgM) against CD11b (a gift from Dr Robert Todd, University of Michigan) was used in some studies.

Neutrophil adhesion to human umbilical vein endothelial cells and human fibroblasts. Neutrophils (5 × 10⁶ cells in Hank’s balanced salt solution [HBSS]) were labeled with [3H] (60 µCi) for 60 minutes at 37°C, washed twice in HBSS containing 0.5% human albumin (Baxter Healthcare, Hyland Division, Glendale, CA).
(HBSS-A), and resuspended at a concentration of $2 \times 10^7$ cells/mL in HBSS. The adhesion of neutrophils to human umbilical vein endothelial cells (HUVEC) and fibroblasts was assayed as previously described. In brief, HUVEC or cultured human fibroblasts were grown to confluence in a 24-well dish (Costar, Cambridge, MA) to which was added neutrophil cell suspension (100 µL), serum or HBSS-A (100 µL), and quinine-HCl or HBSS-A (100 µL) for 15 minutes at 37°C. The monolayers were then washed three times with HBSS-A, the supernatants saved, and the adherent neutrophils and HUVEC then incubated for 10 minutes with 1N sodium hydroxide. The amount of $^{51}$Cr in the combined supernatants and adherent cells was determined with a gamma counter (Beckman Gamma 5500, Irvine, CA) and specific adherence was defined as CPM in supernatants/CPM in supernatants + CPM in adherent cells) $\times 100$.20

Quinine-dependent binding of immunoglobulin to HUVEC. The binding of antibodies to HUVEC was assayed using a modification of an enzyme-linked immunoadsorbent assay (ELISA) technique as previously described.21 HUVEC were grown to confluence in a 96-well dish (Costar). The HUVEC were incubated with 0.1% glutaraldehyde in PBS for 5 minutes at 4°C and washed three times with PBS. HUVEC were then incubated in PBS containing 1% BSA for 1 hour at 20°C, and then serum with or without quinine-HCl was added and the incubation continued for 18 hours at 37°C thereafter. HUVEC were washed three times with PBS, and goat anti-human IgG-alkaline phosphatase diluted 1:1,000 in PBS with 1% BSA for 1 hour at 20°C, and then serum with or without quinine-HCl was added and the incubation continued for 18 hours at 37°C. The amount of $^{51}$Cr in the combined supernatants and adherent cells was determined with a gamma counter (Beckman Gamma 5500, Irvine, CA) and specific adherence was defined as CPM in supernatants/CPM in supernatants + CPM in adherent cells) $\times 100$.20

RESULTS

Antibody testing. The reaction of patient serum with neutrophils was analyzed by flow cytometry and is shown in Fig 2. Reactivity of patient serum added alone to neutrophils (Fig 2B) was no different than the reaction of normal human serum (NHS) alone (Fig 2A) or NHS plus quinine (Fig 2C). However, the addition of quinine to patient serum resulted in strong binding of immunoglobulin to the neutrophils (Fig 2D). Analogously, when patient serum was reacted with platelets or RBCs, no antibodies were detected in serum alone; however, in the presence of quinine, strongly reactive antibodies to platelets and RBCs were identified by immunofluorescence and agglutination assays, respectively (not shown).

Patient serum was found to have quinine-dependent antibodies to T, but not B, lymphocytes. In a flow cytometry assay, patient serum reacted with T lymphocytes from four different donors (mean cell channel number 370 ± 20 [mean ± 1 SD]), but serum alone did not (42 ± 7). NHS and NHS plus quinine did not react with T lymphocytes. Quinine-dependent antibodies to B lymphocytes were not detected in a lymphocytotoxicity assay. Patient serum alone and patient serum plus quinine reacted with B lymphocytes from four of 10 donors tested. NHS alone reacted with B lymphocytes from one of 10 donors and NHS plus quinine did not react with B cells from any of the 10 donors.

The quinine-dependent neutrophil and platelet antibodies were of high titer. In the presence of quinine-HCl (final concentration, 100 µg/mL), patient serum diluted up to 2,048-fold in PBS continued to react with neutrophils, and patient serum diluted 1:30 reacted with neutrophils when as little as 0.1 µg/mL of quinine-HCl was present. Quinine-HCl (500 µg/mL) plus patient serum as dilute as 1:4,096 reacted with platelets in the PIF assay, and at dilution of serum of 1:4 the addition of quinine-HCl at a final concentration as low as 0.25 µg/mL resulted in positive reactions in the PIF assay. Using antiglobulin reagents specific for human IgG or IgM, we found that the quinine-dependent antibodies reacting with platelets and neutrophils were of both types. In the presence of patient serum plus quinine, RBC agglutination occurred even in the absence of antiglobulin reagent, indicating that the RBC antibodies were IgM. In the presence of quinine-HCl (100 µg/mL), patient serum diluted up to 1:32 caused RBC agglutination, and quinine-HCl in concentrations as low as 0.1 µg/mL induced RBC agglutination.

Since quinine and quinidine have similar structure, patient serum was also tested for quinidine-dependent antibodies to neutrophils, platelets, and RBCs. Using PIF, RBC agglutination, and neutrophil flow cytometry, we could detect no quinidine-dependent antibodies.

Patient serum was also tested against blood cells that had been pretreated with quinine. Platelets and RBCs were incubated with quinine for 30 minutes at 37°C, washed three times, and tested with patient serum. No drug-dependent antibody reactions were detected unless additional quinine was added. In contrast, neutrophils pretreated with as little as 1 µg/mL quinine and then thoroughly washed continued to react with patient’s serum in the absence of additional drug.

To determine if the quinine-dependent antibodies against neutrophils, RBCs, and platelets were reacting with similar epitopes, we adsorbed patient serum with RBCs or platelets in the presence of quinine and then reacted it with fresh
quinine plus neutrophils, RBCs, or platelets. Serum that had been adsorbed with platelets plus quinine no longer reacted with platelets, but it continued to react with neutrophils up to a dilution of 1:1,024 and RBCs. After serum was absorbed with RBCs plus quinine, it no longer reacted with RBCs, but serum diluted 1,024-fold continued to react with both platelets and neutrophils. These studies suggest that the quinine-dependent antibodies are reacting with different epitopes on RBCs, platelets, and neutrophils.

**Biochemical studies.** Patient serum was tested for reactivity to platelet antigens in the MAIPA assay. When quinine was added to patient serum, IgG reacted with two platelet membrane GP complexes, namely GPIIb/IIIa and GPIb/IX (Fig 3), but not GPIa/IIa. No reactions occurred with either GP complex in the absence of drug. NHS, with and without quinine, did not react with GPIIb/IIIa, GPIb/IX, or GPIa/IIa.

To determine which neutrophil membrane components the quinine-dependent antibodies recognized as epitopes, immunoprecipitation assays were performed with solubilized 125I surface-labeled neutrophils. In the presence of quinine, patient serum reacted strongly with an 85-Kd protein and weakly with a 130-Kd and a 60-Kd protein (Fig 4, lane D). Patient serum alone (lane C), NHS alone (lane A), and NHS plus quinine (lane B) did not react with any GP of this size. The patient serum plus quinine immunoprecipitated (1) the 85-Kd GP from neutrophils of all 14 donors tested; (2) the 60-Kd GP on neutrophils from 11 of the donors; and (3) the 130-Kd GP on neutrophils from two donors.

Immunoprecipitation was also performed with serum collected on several different days during the hospitalization for the sixth episode. Sera collected on the day of admission (February 5), February 7, February 12, and February 14 were reacted with neutrophils from the same donor in the presence and absence of quinine (Fig 5). All samples tested, including NHS, reacted nonspecifically with an approximately 95-Kd band. On admission, patient serum alone reacted strongly with the 85-Kd protein (Fig 5, lane C) and the reaction was not enhanced by the addition of quinine (Fig 5, lane D). When tested without quinine, sera collected later during the hospitalization reacted weakly or not at all with the 85-Kd protein (Fig 5, lanes E, G, and I), but in the presence of quinine, the sera reacted strongly with this protein (Fig 5, lanes F, H, and J). Serum collected on February 12 and February 14 also reacted weakly with a 60-Kd protein in the presence of quinine.

**Effects of quinine-dependent antibodies on endothelium and neutrophil/endothelial interaction.** The prominent fragmentation hemolysis occurring during two of the patient’s hospitalizations suggested that endothelial damage may have occurred. That the patient’s quinine-dependent antibodies might directly react with endothelial cells was investigated by reacting serum with HUVEC and assaying binding of immunoglobulin using an ELISA. A very weak IgG binding to HUVEC was detected, but no enhancement was provoked by addition of quinine (data not shown). In contrast, marked stimulation of neutrophil aggregation and adhesion to endothelial cells was noted with addition of patient’s serum plus quinine. Thus, as shown by neutrophil aggregometry in Fig 6, NHS did not aggregate neutrophils, whether in the presence or absence of quinine (Fig 6B); nor did patient serum alone (Fig 6C). However, the addition of quinine to patient serum (Fig 6A) resulted in prompt and potent neutrophil aggregation. The quinine-serum-induced neutrophil aggregation was not due to the passive agglutination of neutrophils since (1) aggregation studies repeated with neutrophils that were pretreated with formaldehyde, or with monoclonal antibody Mo 1-17 (CD11b) did not...
induce aggregation (Fig 7B and C); and (2) neutrophils suspended in a calcium- and magnesium-free HBSS did not aggregate when exposed to patient serum plus quinine (data not shown). That quinine-dependent neutrophil aggregation requires IgG was validated in that treatment with staphylococcal protein A, removed the activity from patient serum (Fig 7D). Moreover, neutrophil aggregation probably required activation of complement components by antibody-antigen complexes, since serum decomplemented by incubation at 56°C for 30 minutes failed to aggregate neutrophils in the presence of quinine (Fig 7E), despite the fact that significant antibody binding to neutrophils could be detected by flow cytometry under these conditions (data not shown).

Quinine/antibody-dependent neutrophil activation was manifest not only by self-adhesion (aggregation), but also produced neutrophil/endothelial adhesion. We analyzed the ability of serum to induce adherence of neutrophils to cultured HUVEC (Fig 8), and found such adhesion no different in the presence of patient serum or pooled human serum (PHS). Moreover, addition of quinine alone or to PHS had no effect on neutrophil adhesion. In contrast, addition of quinine to patient serum resulted in markedly increased adhesion of neutrophils to HUVEC from 20% to nearly 80% (Fig 8). This hyperadhesivity also resulted in easily discerned clumping of neutrophils on the cultured HUVEC monolayers (Fig 9A and B). The role of activated complement in this phenomenon was suggested, since addition of quinine to heat-decomplemented patient serum did not induce neutrophil clumping or amplify adhesion of neutrophils to HUVEC (data not shown).

Patient serum plus quinine also induced neutrophil adhesion to fibroblast monolayers. When neutrophils were incubated with patient serum, neutrophil adherence was 32%, but, in the presence of quinine, neutrophil adherence...
Fig 7. Quinine-induced neutrophil aggregation is dependent on IgG. (A) Patient serum, (B) patient serum plus 2% formaldehyde, or (C) patient serum plus monoclonal antibody Mo 1-17 (CD11b) (5 μg/mL) were incubated with neutrophils at 37°C for 1 minute. Quinine (500 μmol/L) was added and light transmission was measured for 3 minutes. Staphylococcus protein A-treated patient serum (D) and heat-inactivated patient serum (E) were incubated with neutrophils at 37°C for 1 minute, and quinine was added.

Fig 8. Patient serum plus quinine (500 μmol/L) induces neutrophil adhesion to HUVEC. Neutrophils were labeled with 51Cr and were incubated with HUVEC and patient serum or PHS in the presence or absence of quinine. HUVEC were washed to remove nonadherent cells and the percent of adherent neutrophils measured.

DISCUSSION

We recently demonstrated that patients with quinine-induced thrombocytopenia can possess at least two distinct drug-dependent antibodies, one reactive with GPIb/IX and the second with GPIIb/IIIa.22 The present results are similar in that the patient’s quinine-dependent platelet antibodies reacted with both GPIb/IX and GPIIb/IIIa. Extending our earlier work by immunoblotting, Pfueller et al described four patients with quinine-dependent antibodies that variably reacted with GPIb, GPIIb, GPIIa, GPIX, and/or an unidentified 57-Kd protein missing in Bernard-Soulier syndrome platelets.23 The MAIPA assay used in the present study would not have detected the 57-Kd protein, so we cannot exclude the possibility that our patient may have also produced antibodies to this protein. More recently, Visentin et al used immunoprecipitation and immunoblotting to demonstrate the presence of quininedependent antibodies specific for the GPIIb/IIIa complex or GPIIb alone or GPIIIa alone among eight patients with drug-induced thrombocytopenia.24
Our patient seems unique from others reported with quinine-dependent cytopenias in that virtual agranulocytosis was a prominent and consistent feature of her six episodes. In fact, ANC levels of virtually 0 were noted for several days during each of her hospitalizations (Fig 1). We are unaware of previous characterization of antineutrophil antibodies in quinine-hypersensitive patients. In the present studies, we found our patient’s quinine-dependent antibodies reacted strongly with an 85-Kd and weakly with a 60-Kd protein. While patients with quinidine-dependent agranulocytosis25,26 and quinidine-dependent thrombocytopenia and leukopenia27 have been reported, the neutrophil antigens to which these antibodies were directed have not been identified. Serum from a previously reported patient immunoprecipitated GPIb and a platelet membrane GP of 22 Kd, but did not immunoprecipitate any neutrophil membrane GP.27 The patient in that study was similar to our patient in harboring two distinct antibodies, one reacting with platelets and one reacting with neutrophils,27 but the latter was not further characterized.

In contrast to quinidine hypersensitivity, rare patients with quinine-dependent antibodies to platelets have been recently reported to develop full-blown HUS or severe disseminated intravascular coagulopathy.8,9 Our patient experienced one episode of severe HUS, requiring hemodialysis, and more mild renal damage during her last hospitalization. While the exact pathophysiology of HUS is not certain, endothelial cell damage is likely to be an important factor in this syndrome. Endothelial cells contain membrane GPs immunologically similar to platelet GPIb28,29 and also to the integrin receptor, GPIIa30; therefore, HUS in a patient like ours might be due to quinine-dependent antibodies that react with antigens expressed both by platelets and endothelial cells. Indeed, Pfueller et al have characterized a quinine-dependent antibody that reacts with platelet membrane GPIIIa, as well as with endothelial cells.31 While our patient manifested quinine-dependent antibodies directed against multiple blood cells, including at least one to the platelet GP IIb/IIIa complex, we were unable to demonstrate direct, specific reactivity of patient IgG with cultured endothelial cells.

While the patient’s quinine-dependent antibody to RBCs likely induced some hemolysis, the present studies do suggest that quinine-dependent antibodies could have less directly contributed to our patient’s episodes of renal failure and fragmentation hemolysis by activating neutrophils. Generation of C5a during hemodialysis has been shown to cause neutrophil aggregation and in vivo can result in neutropenia and pulmonary edema due to activated neutrophil-mediated damage to pulmonary endothelium.32 Moreover, the inadvertent transfusion of blood products containing neutrophil-specific, complement-activating antibodies can also result in rapid high-protein pulmonary edema (adult respiratory distress syndrome).33,34 The quinine-dependent antibodies in our patient stimulated neutrophils and caused their complement-dependent adhesion to cultured endothelial in previous studies from our laboratory35 and others,36 similarly adhesive, activated neutrophils, readily damaged cultured endothelial cells. From these considerations, we find it attractive to speculate that the ingestion of quinine by our patient provoked neutrophil-mediated vascular damage causing an HUS-like picture. In this regard, we find it interesting that Walters et al have reported that disease outcome in childhood HUS correlates with neutrophil counts at the time of presentation;37 patients with higher neutrophil counts have a worse outcome. Although our patient developed HUS during her initial episode, when most recently challenged with quinine, she developed hemolysis, thrombocytopenia, and agranulocytosis, but only mild renal impairment and elevated fibrin degradation products. It is uncertain why she did not develop full-blown HUS during each quinine exposure. We speculate that with more recent and frequent exposures to quinine, her quinine-dependent antibody concentrations may have increased to levels that prompted such abrupt clearance of neutrophils that insufficient numbers were available to mediate severe endothelial cell damage.

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