Severe thrombocytopenia developed in a patient with acquired immunodeficiency syndrome during treatment with intravenous pentamidine for Pneumocystis carinii pneumonia. The patient's bone marrow contained adequate numbers of megakaryocytes, suggesting peripheral platelet destruction. Platelet counts ranged between less than 3 and 20 × 10^9/L for 2 weeks despite cessation of pentamidine, platelet transfusions, high-dose intravenous IgG, and 2 mg/kg/d prednisone. Thereafter, the platelet count increased to prepentamidine levels (95 × 10^9/L), permitting rapid withdrawal of steroids. Testing by immunofluorescence disclosed a high-titer, pentamidine-dependent IgG antibody in the patient’s acute-phase serum that almost entirely disappeared by the time the patient’s platelet count returned to baseline levels. This antibody reacted only with platelet glycoprotein (GP) IIb/IIIa as shown by antigen-capture enzyme-linked immunosorbent assay using monoclonal antibodies specific for various GPs, and was absorbable by normal, but not by GPIIb/IIIa-deficient platelets (from a patient with Glanzmann’s thrombasthenia). The pentamidine-dependent antibody could not be demonstrated by immunoprecipitation using the patient’s serum and 125I-labeled normal platelets, although a separate pentamidine-independent antibody was detected by this method. This latter antibody reacted with two GPs having molecular weights consistent with GPIIb/IIIa, and was present in postrecuperation as well as acute-phase sera. However, only the pentamidine-dependent antibody was temporally associated with the severe thrombocytopenia. Therefore, we believe that these studies demonstrate, for the first time, that intravenous pentamidine therapy can provoke formation of drug-dependent antibodies that induce immunologic thrombocytopenia.

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From the Department of Laboratory Medicine and Pathology, University of Minnesota, and the Department of Medicine, VA Medical Center, Minneapolis, MN.

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PENTAMIDINE (4,4’-diamidophenoxypentane), an antiprotozoal agent, has been used in the treatment of Pneumocystis carinii pneumonia (PCP) since the late 1950s. As a result of the recent acquired immunodeficiency syndrome (AIDS) pandemic with its frequently accompanying PCP infections, the use of this drug has increased dramatically. Although parenterally administered pentamidine is associated with a variety of side effects, severe thrombocytopenia has been observed relatively infrequently. Thus, in a recent study of 34 AIDS patients treated with parenteral pentamidine, 6 (18%) developed moderately severe thrombocytopenia (> 25 × 10^9/L). In two reports of more severe pentamidine-associated thrombocytopenia, efforts to detect pentamidine-dependent antibodies were unsuccessful.

Immune-mediated thrombocytopenia associated with drug-dependent antibodies has been well-documented for quinine, quinidine, and more than 100 other low molecular weight compounds and therapeutic agents. The platelet glycoprotein (GP) Ib/IX complex was first identified as the attachment site of quinine/quinidine-dependent antibodies. However, more recently, it has been shown that some of these antibodies may also bind to epitopes on the GPIIIa complex. In this report we describe a patient with AIDS and PCP who, because of development of sulfonamide hypersensitivity, was treated with intravenous (IV) pentamidine. During the course of this therapy, he developed sudden, severe thrombocytopenic purpura accompanied by high-titer, pentamidine-dependent antibodies specific for GPIIb/IIIa.

MATERIALS AND METHODS

Case report. A 37-year-old, human immunodeficiency virus (HIV)-positive Caucasian man (RH) with documented PCP was treated (Fig 1) with IV followed by oral trimethoprim/sulfamethoxazole (Septra, Burroughs Wellcome Co, Research Triangle Park, NC). The pneumonia gradually resolved. However, on day 11 of the planned 3-week course of therapy (Fig 1, day 0), the patient developed a diffuse maculopapular rash consistent with sulfonamide allergy. He was immediately hospitalized, at which time his platelet count was 122 × 10^9/L, the white blood cell count (WBC) 8.4 × 10^9/L, and the hemoglobin (Hgb) level 11.0 g/dL. Septra was discontinued, and IV pentamidine therapy was started the next day. During the first 8 days of pentamidine treatment, the patient’s rash gradually faded, and his platelet counts ranged between 60 and 70 × 10^9/L. However, on day 9 of pentamidine (Fig 1, day 10), the patient was noted to have developed widespread petechiae and ecchymoses, and his platelet count had decreased to less than 3 × 10^9/L. A bone marrow examination disclosed normal numbers of megakaryocytes. Despite discontinuation of pentamidine (Fig 1, day 12), transfusion of pooled random donor platelet concentrates, and treatment with oral prednisone (2 mg/kg) and high-dose IV gamma-globulin (IVIG), platelet counts remained between less than 3 and 20 × 10^9/L for the next 2 weeks. Thereafter, as the platelets gradually increased to baseline levels (95 × 10^9/L), the patient was rapidly
weaned off steroids. He was discharged after 5 weeks of hospitalization.

**Platelet antibody studies.** All blood samples were obtained after receiving informed consent and with the approval of the University of Minnesota Committee on the Use of Human Subjects in Research. Three separate serum (S) samples from patient RH were available for study: (1) S1, obtained 1 day before pentamidine was discontinued (Fig 1, day 11); (2) S2, obtained 3 days after pentamidine was discontinued (Fig 1, day 15); and (3) S3, obtained 27 days after pentamidine therapy (Fig 1, day 39). Platelet antibodies were sought in the patient’s sera using the platelet-suspension immunofluorescence test (PIFT) and fluorescence isothiocyanate (FITC)-labeled antihuman IgG or IgM [F(ab')2 fragments], as previously described.

GP specificities of antibodies detected by PIFT were determined using a modification13 of the monoclonal antibody (MoAb)-specific immobilization of platelet antigens (MAIPA) assay.15 Whole blood from normal volunteers was anticoagulated with 7.8 mmol/L EDTA and centrifuged at 150g for 10 minutes to prepare platelet-rich plasma (PRP). Platelets were isolated from PRP by centrifugation at 1,000g for 7 minutes and washed twice in phosphate-buffered saline (PBS)-EDTA (0.1 mol/L Na2HPO4, 0.15 mol/L NaCl, 8 mmol/L EDTA, pH 7.4). Platelets (5 x 109) were incubated for 30 minutes at 37°C with 30 to 50 mL of patient or normal human (transfused, AB, male) serum in the presence and absence of 1 mmol/L pentamidine. Serum samples and platelets were separated by centrifugation for 5 minutes at 13,000g. The absorbed sera were then tested in the MAIPA assay for residual pentamidine-dependent antibody activity.

**Immunoprecipitation.** Washed platelets (1 x 10⁸) were labeled with 1 mCi Na125I (NEN/Dupont, Boston, MA) in PBS, pH 7.8, and immunoprecipitation of platelet GPs was performed as previously described. Briefer, 5 x 10⁶ 125I-labeled platelets were solubilized for 30 minutes at 37°C in 0.5 mL TBS (20 mmol/L Tris, 145 mmol/L NaCl, pH 8.6) containing 0.05% Tween (TBST), 1% Triton X-100, and 0.2 mmol/L phenylmethylsulfonyl fluoride in the presence of 5 mmol/L EDTA or 1 mmol/L CaCl₂. Solubilized platelets (100 µL) were incubated for 1 hour at room temperature with either 50 µL patient or normal serum (obtained from a nontransfused type AB male donor) in the presence or absence of 300 µmol/L pentamidine (final concentration) pentamidine isethionate (Fujiwawa Pharmaceutical Co, Deerfield, IL). Platelets were then washed and further incubated at 4°C with rabbit antihuman Igs (γ- or μ-chain-specific; Jackson Immunoresearch, West Grove, PA) coupled to alkaline phosphatase. The wells were then washed and the enzyme reaction developed with para-nitrophenylphosphate (Sigma Chemical Co, St Louis, MO) and read at 405 nm with a Biokinetics Reader EL 340 (Bio-Tek Instruments, Winooski, VT). Pentamidine (300 µmol/L) was present in all washes and in the solubilization buffer for those samples initially incubated with drug. In control experiments, serum from a patient with documented immune thrombocytopenia due to a quinine-dependent antibody16 was used for comparison.

![Fig 1. Clinical course of patient RH during pentamidine treatment. Hospitalization occurred on day 0. Transfusions included RBCs (R) and pooled random donor platelet concentrates (]).](image-url)
RESULTS

Detection of pentamidine-dependent antibodies. High-titer IgG pentamidine-dependent antibodies were detected by PIFT in both acute-phase serum samples (S1 and S2). Positive results with S1 must be interpreted with caution because agglutination can affect platelet fluorescence. Antibody titers were markedly reduced in postrecovery serum (S3) (Table 1). The reactivity of these sera with the patient’s postrecovery (autologous) platelets was identical to that observed with panel platelets, ie, S1 and S2 (to a lesser degree) reacted with platelets in the absence of added pentamidine, presumably due to the presence of residual drug. However, their reactivities were strongly enhanced in the presence of added drug. S3 reacted very weakly and only when pentamidine was added. No drug-dependent antibody activity was detected in the patient’s sera using FITC-labeled antihuman IgM, nor was pentamidine-dependent IgG or IgM binding to platelets detected with normal human serum (not shown). To address the possibility that the pentamidine-dependent IgG activity was due to nonspecific effects of AIDS- and/or IVIG-induced hypergammaglobulinemia, PIFT and MAIPA tests were performed with serum from a different thrombocytopenic AIDS patient who was also receiving IV pentamidine. Both tests were negative for platelet antibodies in the presence and absence of pentamidine (not shown). Moreover, because S1 of patient RH was collected before infusion of IVIG, passively administered antibody could not account for the positive results obtained.

Because the patient had been transfused with 2 U of red blood cells (RBCs) 20 days before the onset of acute thrombocytopenia (Fig 1), the possibility was considered that he had developed posttransfusion purpura. However, none of the patient’s sera tested positive for anti–HPA-1a, -1b, -2b, -3a, -3b, -4a, or -5b by PIFT and/or the MAIPA assay. Thus, it is highly unlikely that posttransfusion purpura was the etiology of severe thrombocytopenia.

Characterization of pentamidine-dependent antibody binding site(s) by MAIPA. To explore possible attachment site(s) on platelets of the pentamidine-dependent antibody, we used a panel of MoAbs directed against various platelet membrane GPs (Fig 2A and B). Strong pentamidine-dependent antibody activity was detected in both S1 and S2 when

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<th>Table 1. Detection of Pentamidine-Dependent Antibodies by PIFT</th>
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Immunofluorescence was scored from 1+ (weakest) to 4+ (strongest). S1 was obtained while RH was receiving pentamidine; S2 and S3 were collected 3 and 27 days, respectively, after discontinuation of pentamidine.

Abbreviations: Pent, 300 μmol/L pentamidine; Aggl, agglutinated; VD, very dull; ND, not done.

Fig 2. Characterization of pentamidine-dependent antibodies by MAIPA assay. (A) and (B) show that acute-phase S1 and S2, respectively, from patient RH were reacted with intact platelets in the presence of 300 μmol/L (final concentration) pentamidine (●) or PBS (□). (C) Serum from another patient having documented quinine-dependent antibodies16 was reacted with the same intact platelets as those used in (A) and (B) in the presence of 300 μmol/L (final concentration) quinine (●) or PBS (□). Platelets were then solubilized and platelet GP-antibody complexes captured in microwells by the various MoAbs indicated on the abscissa. After capture, drug-dependent antibody complexes were detected using antihuman IgG conjugated to alkaline phosphatase and paranitrophosphosphate substrate. The optical density was read at 405 nm.

the GPIIb/IIIa complex was captured with AP3 or SZ21 (anti-GPIIb/IIIa), or 10E5 (anti-GPIIb/IIIa), whereas weaker reactivity was detected using ZZ22 (anti-GPIIb) and P2 (anti-GPIIb/IIIa). However, no pentamidine-dependent antibody activity was detected when GPib/IX or GPIa/IIa complexes were captured by their respective MoAbs. These results contrasted with the pattern of reactivity observed
with serum from a patient with quinine-induced thrombocytopenia. It reacted strongly with P2, as well as with epitopes on the GPIb/IX complex (Fig 2C). In the absence of added pentamidine, S1 displayed modest antibody reactivity with various MoAbs. This was almost certainly attributable to the high pentamidine levels in S1, which was obtained while the patient was still receiving the drug. By MAIPA, no platelet antibody reactivity was detected in the patient’s convalescent serum (S3) or in normal human serum regardless of whether pentamidine was present. Because pentamidine had no demonstrable effect on the binding of anti–HPA-la to HPA-la-positive platelets (not shown), it is unlikely that pentamidine was simply enhancing the binding of a drug-independent antibody to GPIIb/IIIa.

In an effort to confirm the GPIIb/IIIa binding specificity of the pentamidine-dependent antibody, S2 was absorbed with normal or GT platelets in the presence and absence of 1 mmol/L pentamidine and tested by MAIPA assay for residual antibody activity. More than 90% of the pentamidine-dependent antibody was absorbed by 4 × 10⁷ normal platelets/mL, whereas no observed decrease in pentamidine-dependent antibody activity occurred when the absorption was performed with the same concentration of GT platelets (not shown). In the absence of drug, pentamidine-dependent antibody was absorbed by neither platelet type.

Radioimmunoprecipitation studies. Further characterization of the pentamidine-dependent antibody was attempted using 125I-labeled normal platelets previously solubilized in the presence of EDTA or Ca²⁺ (to dissociate or retain GPIIb/IIIa complex formation, respectively), and then reacted with S1 in the presence and absence of 300 μmol/L pentamidine. Two bands were precipitated having estimated molecular weights of 120 and 90 kD, respectively. These results were unaffected by the presence of pentamidine or by solubilization of platelets in EDTA (Fig 3, lanes 1, 2, 11, and 12). These bands were consistent in molecular weights with those obtained for GPIIb and GPIIIa precipitated by AP3 and 10E5 (lanes 5 and 6, respectively). Essentially complete dissociation of the GPIIb/IIIa complex was achieved by platelet solubilization in EDTA, evident by the absence of detectable bands precipitated by 10E5 (GPIIb/IIIa complex-specific; lane 7). Results identical to those shown in Fig 3 (lanes 1 and 2) were obtained using S2 and S3 (not shown).

DISCUSSION

These studies document the sudden appearance of life-threatening thrombocytopenic purpura in a patient with AIDS after the initiation of daily IV pentamidine therapy. Simultaneously, a high-titer, pentamidine-dependent, antiplatelet antibody was demonstrable in the patient’s serum that almost entirely disappeared by the time the acute thrombocytopenia resolved. We believe these findings demonstrate, for the first time, that pentamidine can elicit formation of drug-dependent platelet antibodies that may result in life-threatening thrombocytopenia.

By MAIPA assay, the pentamidine-dependent IgG antibody was found to exclusively react with GPIIb/IIIa, with strong reactions being observed when the GPIIb/IIIa complex was captured by the MoAbs AP3 and SZ21 (anti-GPIIIa) as well as by 10E5 (anti-GPIIb/IIIa) (Fig 2). In contrast, markedly weaker reactions occurred with SZ22 (anti-GPIIb) and P2 (anti-GPIIb/IIIa). This finding suggests that the pentamidine-dependent antibody and MoAbs SZ22 and P2 bind to the same, or closely proximal, epitopes of GPIIb or the GPIIb/IIIa complex. These findings differed markedly from those observed with a quinine-dependent platelet antibody that reacted strongly with GPIIb/IIIa captured by P2 or SZ22 (Fig 2). The pattern of reactivity of the pentamidine-dependent antibody more closely resembled that of two vancomycin-dependent antibodies that showed specificity for GPIIb/IIIa. Strong independent evidence demonstrating GPIIb/IIIa specificity of the pentamidine-dependent antibody was absorption of this antibody from patient’s serum by normal, but not GT, platelets in the presence of drug.

Using immunoprecipitation, we were surprised to find...
that no pentamidine-dependent bands could be detected in either of the acute-phase serum samples (S1 or S2). Rather, two pentamidine-independent bands, having molecular weights consistent with GPIIb/IIIa, were found (Fig 3, lanes 1, 2, 11, and 12). This same pentamidine-independent antibody activity was present in serum sample S3 obtained after the patient’s platelet count had returned to baseline levels. S3 contained little, if any, pentamidine-dependent antibody by PIFT and MAIPA assays. We interpret these findings to indicate the presence in the patient’s acute-phase sera of two separate antibody populations capable of binding to GPIIb and/or IIIa. The pentamidine-dependent antibody was clearly detected by immunofluorescence and MAIPA, but not by immunoprecipitation; in contrast, the drug-independent antibody was easily demonstrable only by immunoprecipitation. Other investigators have reported drug-independent antibodies accompanying drug-dependent antibodies in patients with thrombocytopenia provoked by quinidine, acetaminophen, and phenazopyridine. In the present study, we are uncertain as to why both antibody populations were not clearly detected by each of the methods used. In the immunoprecipitation experiments, it is possible that binding of the drug-independent antibody masked detection of the pentamidine-dependent antibody. Alternatively, the drug-dependent antibody may be primarily IgG3, a subclass poorly bound by protein A. We have no satisfactory explanation why the drug-independent antibody was not detected by PIFT and the MAIPA assay. Nevertheless, the data strongly argue that the acute thrombocytopenic episode was provoked by the pentamidine-dependent IgG antibody, whereas the drug-independent antibody may be similar to the anti-GPIIb/IIIa platelet antibodies found by some investigators to be present in HIV-infected patients.

Given that thrombocytopenia (usually of a mild to moderate degree, i.e., >30 × 10³ platelets/L) is very frequently seen in AIDS patients, physicians must not overlook the possibility of a drug-mediated, immunologic process that could respond promptly to discontinuation of the offending drug. It seems clear that pentamidine, when administered IV, is yet another agent that should be added to the ever-expanding list of compounds capable of causing life-threatening thrombocytopenia. At the present time, there is no evidence that inhalational pentamidine is capable of eliciting a similar process; however, this theoretic possibility cannot be excluded.

ACKNOWLEDGMENT

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Severe thrombocytopenia in an acquired immunodeficiency syndrome patient associated with pentamidine-dependent antibodies specific for glycoprotein IIb/IIia

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