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Case Report

Acquired Amegakaryocytic Thrombocytopenia Purpura in a Rhesus Macaque (*Macaca mulatta*)

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A 10-y-old multiparous rhesus macaque presented for an annual routine physical examination. Clinically, the animal had pale mucous membranes, petechial and ecchymotic hemorrhages in multiple sites, and a laceration at the tail base. Severe pancytopenia was noted on hematologic evaluation. The monkey was seronegative for SIV, simian T-lymphotropic virus, simian retrovirus type D, and *Macacine herpesvirus* 1. Bone marrow evaluation revealed a paucity of megakaryocytic precursors in a hypercellular marrow with marked erythroid hyperplasia. In light of these findings, the diagnosis was acquired amegakaryocytic thrombocytopenia purpura. Due to the poor prognosis of the syndrome and clinical deterioration of the monkey, euthanasia was elected. A definitive cause of the thrombocytopenia was not identified; however, the syndrome may have developed secondary to a recent spontaneous abortion. To our knowledge, this case represents the first reported observation of acquired amegakaryocytic thrombocytopenia purpura in a rhesus monkey.

Abbreviation: AATP, acquired amegakaryocytic thrombocytopenia purpura.

Case Report

A 10-y-old multiparous rhesus macaque (*Macaca mulatta*; weight, 5.9 kg) presented for a routine physical examination at the AAALAC-accredited Michale E Keeling Center for Comparative Medicine and Research facility of the University of Texas MD Anderson Cancer Center. The monkey was part of an SPF breeding colony and serologically negative for SIV, simian T-lymphotropic virus, simian retrovirus type D, and *Macacine herpesvirus* 1. The monkey was vaccinated for measles and tetanus and had a recent negative intradermal skin test for tuberculosis. The animal was on an IACUC-approved protocol and was managed in accordance with US Department of Agriculture Animal Welfare Regulations[2] and the *Guide for the Care and Use of Laboratory Animals*.[12]

As part of the breeding colony, the monkey we present was housed in a unimale–multifemale group in outdoor pens. Every monkey in the group was visually observed at least twice daily by the animal care staff and at least once daily by a veterinarian. Annually, every monkey has a physical examination that includes viral testing, tuberculosis testing, and routine blood work (CBC, complete biochemical panel). They were fed a commercial monkey diet (Purina Cu Lab Hi-F Primate Diet, PMI, St Louis, MO) with additional food enrichment at least twice daily. Additional enrichment was provided in the form of manipulanda, visual stimulation, or auditory stimulation and combinations thereof.

The initial physical examination revealed pale mucous membranes, with petechiae and ecchymoses along the face, in the axillary regions bilaterally, and along the plantar surface of the lower left leg. An open laceration at the tail base from recent trauma was associated with extensive bruising up to the right illial wing. In addition, a 3 × 3 cm raised hematoma had developed on the dorsal surface of the right wrist. A grade II/VI holosystolic left-sided murmur with tachycardia was noted on thoracic auscultation. Abdominal ultrasonography did not reveal any major organ abnormalities. Ophthalmic examination was unremarkable. An initial (day 1) CBC (Advia 120 Hematology Analyzer, Siemens Healthcare Diagnostics, Tarrytown, NY) and manual reticulocyte count demonstrated severe anemia with mild regeneration, severe leukopenia characterized by a severe neutropenia with a degenerative left shift back to the metamyelocyte stage, and severe thrombocytopenia. Notable alterations in RBC morphology included mild schistocytosis, moderate poikilocytosis, mild elliptocytosis, moderate anisocytosis, severe hypochromasia, and moderate polychromasia. A serum biochemistry panel was within normal limits. A coagulation panel revealed normal activated partial thromboplastin and prothrombin times, elevation of fibrinogen, and decreased antithrombin III percentage. The elevation of fibrinogen likely represented inflammation, and the decreased AT III percentage was attributed to hemorrhage and widespread clotting. Despite erythroid regeneration demonstrated by an increase in reticulocytes and moderate polychromasia, a blood transfusion was elected based on the severity of the anemia and the ongoing blood loss. (Table 1). After major and minor cross-matching with suitable donors, the monkey was transfused with 60 mL whole blood. Although a nidus of infection was not identified, enrofloxacin (10 mg/kg IM twice daily) was administered in light of the severity of the degenerate left shift. Taking into account the pancytopenia and clinical presentation, initial differential diagnoses included immune-mediated thrombocytopenia with severe hemorrhage and idiopathic aplastic anemia.

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Table 1. Hematologic findings from a rhesus monkey with severe thrombocytopenia and anemia.

	Day 1	Day 2	Day 4	Reference interval
RBC count (x 10^6)	1.06	1.49	1.42	5.34–6.16
HCT (%)	9.81	12.1	15.8	37.7–42.9
Hemoglobin (g/L)	3.27	3.97	5.10	12.1–13.7
MCV (fl)	92.2	80.9	78.8	66.8–74
MCH (pg)	30.8	26.6	25.4	21.1–23.7
MCHC (g/dL)	33.4	32.9	32.2	31.3–32.5
Absolute reticulocyte count (x 10^8/mL)	424	596	804	61.1–190.3
Platelets (x 10^9/L)	15.1	52.4	23.9	260–361
WBC count (x 10^9/L)	2.01	1.43	1.42	7–13.6
Neutrophils (x 10^9/L)	1.17	0.69	0.61	3.74–10.06
Lymphocytes (x 10^9/L)	0.82	0.72	0.71	0–8.32
Monocytes (x 10^9/L)	0	0.03	0.03	0–0.62
Eosinophils (x 10^9/L)	0	0	0.07	0–0.32
Metamyelocytes (x 10^9/L)	0.02	0	0	0
Nucleated RBC per 100 WBC	0	1	0	0
Prothrombin time (s)	9.6	ND	9.9	10.1–12.9
Fibrinogen (mg/dL)	664	ND	446	ND
Activated partial thromboplastin time (s)	19.4	ND	20.5	34.4–36.6
Antithrombin III (%)	72	ND	89	ND

ND, not determined

On day 2, the erythrogram and thrombogram showed slight improvement, evidenced by mild elevations in the hematocrit, RBC count, and platelet count (Table 1). A second blood transfusion (60 mL) was completed. Dexamethasone (1 mg/kg IM) was given to address the possibility of an immune-mediated process. To improve the antibiotic spectrum and to increase bacterial killing in the face of iatrogenic immunosuppression (dexamethasone administration), the antibiotic was changed to imipenem–cilastatin. To fully evaluate the pancytopenia, a bone marrow aspirate was obtained from the left femur. Ketamine (10 mg/kg IM) was used for induction of anesthesia, and isoflurane was used to maintain a surgical plane of anesthesia. Buprenorphine (0.05 mg/kg IM) was given for pain control after bone marrow aspiration.

The bone marrow was markedly hypercellular with a myeloid-to-erythroid ratio of approximately 1:3. Given the severity of the thrombocytopenia, the megakaryocytic series was profoundly hypoplastic, with approximately one mature megakaryocyte per every 5 to 10 high-power fields. The erythroid series exhibited marked hyperplasia with complete maturation and a large increase in the storage pool of rubricytes and metarubricytes (Figure 1 A). This response was considered appropriate, given the severity of the blood loss and the magnitude of the anemia. The myeloid series displayed a slight increase in the number of early precursors and a mild decrease in the storage pool of band and segmented neutrophils. In light of the severe neutropenia, these findings suggested that neutrophil production was inadequate or that myelogenesis had not yet increased to a level sufficient to compensate for an increased peripheral demand for neutrophils. Mild lymphocytosis and moderate plasmacytosis with an occasional Mott cell indicated nonspecific immune stimulation (Figure 1 B). Iron stores were adequate.

By day 3, the monkey had lost 285 g of body weight and had developed vaginal hemorrhage. In addition, a fundic examination demonstrated retinal hemorrhages. A large hematoma was present in one of the femoral triangles recently used for venipuncture. Imipenem–cilastatin was continued prophylactically. The absence of megakaryocytopoiesis suggested a primary differential diagnosis of acquired amegakaryocytic thrombocytopenia purpura (AATP).

On day 4, a CBC (Table 1) revealed a mild improvement in the erythrogram, but the leukogram and platelet count had continued to decline. Biochemical abnormalities included elevations in creatinine kinase, ALT, AST, and LDH, consistent with muscular trauma induced by repeated venipuncture and intramuscular injections. The macaque was mildly hypernatremic and hyperchloremic, consistent with poor water intake and mild dehydration. Urinalysis from a voided urine sample was unremarkable. The monkey was euthanized in light of a poor prognosis and continued clinical deterioration.

At necropsy, the monkey was thin with scant subcutaneous and mesenteric fat. A small amount of blood was present around the vagina, and small bite wounds were present around the base of the tail. The abdominal cavity contained approximately 5 mL of straw-colored fluid. Cytologic evaluation of the fluid revealed a modified transudate with a mild lymphocytosis that was interpreted as chronic inflammation or irritation within the abdominal cavity. The liver had a prominent reticular pattern, and the gall bladder had 2 small foci of hemorrhage. Petechial hemorrhages were noted on the urinary bladder and remnants of the thymus. A large hematoma was present on the right upper thigh. Histologically, the bone marrow was markedly hyperplastic, with a decreased myeloid-to-erythroid ratio and a severe depletion of megakaryocytes (Figure 2 A and B).

Discussion

Naturally occurring pancytopenia in SPF rhesus macaques is an uncommon presentation. Most reports of pancytopenia in

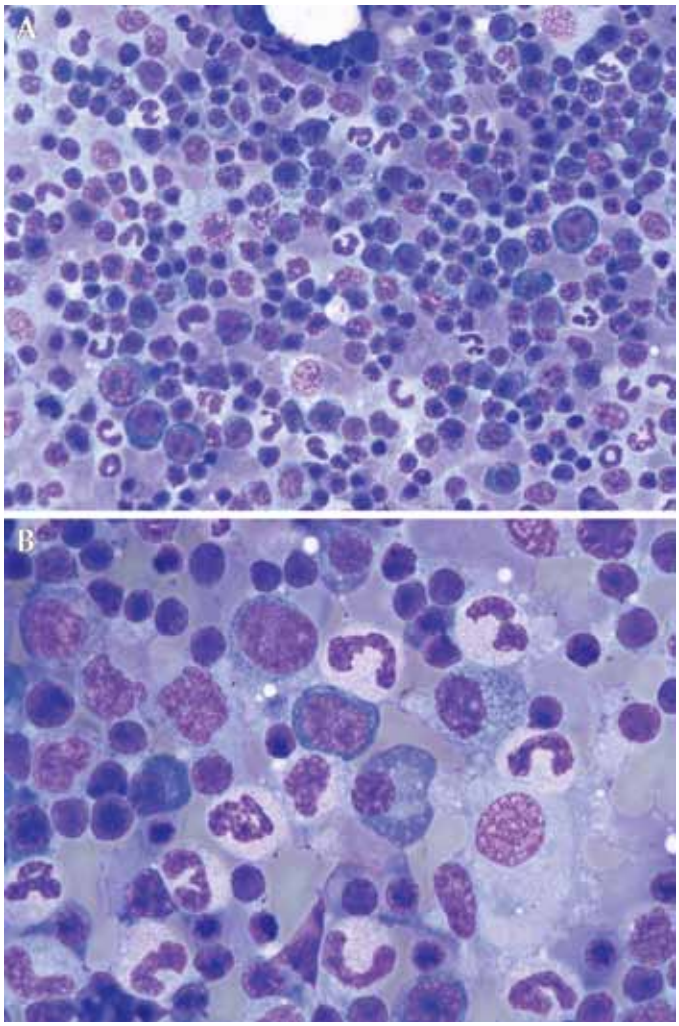

Figure 1. Bone marrow aspirate from the femur of an adult female rhesus macaque. (A) Marked erythroid hyperplasia and absence of megakaryocytes. Modified Wright–Giemsa stain; magnification, 500×. (B) Moderate plasmacytosis with occasional Mott cells and mild lymphocytosis. Modified Wright–Giemsa stain; magnification, 1000×.

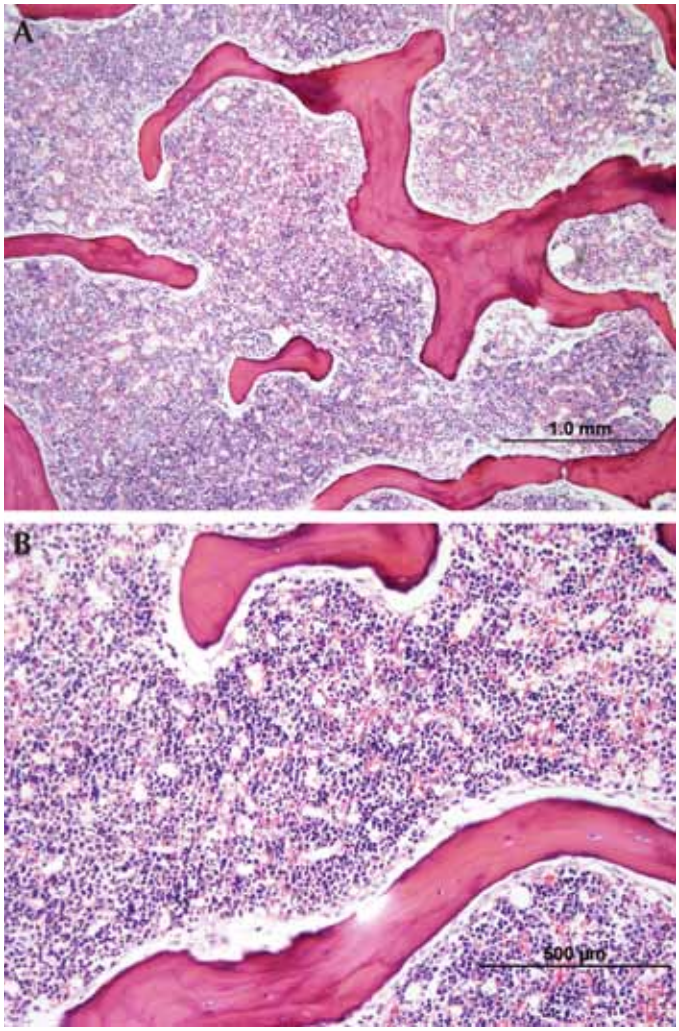

Figure 2. Histologic section of bone marrow obtained from the sternum of an adult female rhesus macaque at the time of necropsy. The sample is markedly hypercellular with a decreased myeloid-to-erythroid ratio and a paucity of megakaryocytes. Hematoxylin and eosin stain; bar: 1.0 mm (A); 0.5 mm (B).

monkeys can be contributed to experimental manipulation (for example drug toxicity, radiation exposure) or infection with SRV.[18] The monkey we present was research naïve, negative for retroviruses, and had never been irradiated or received any experimental drugs. Given the clinical presentation and hematologic findings, initial differential diagnoses included immune-mediated thrombocytopenia complicated by severe hemorrhage and idiopathic aplastic anemia, although the degree of erythrocyte regeneration would have been less consistent with aplastic anemia. In addition, aplastic anemias typically are characterized by a hypocellular bone marrow, whereas in the present case, the marrow was hypercellular, with a specific decrease in megakaryocyte numbers and a possible defect in myelogenesis.[26,27]

The marked depletion of megakaryocytic precursors was consistent with an uncommon condition in humans known as AATP.[10,11] In the veterinary literature, a single case report in a dog and a case series of 4 dogs with this syndrome have been described.[15] Overall, AATP is characterized by severe thrombocytopenia (fewer than 20,000 cells per cubic millimeter) with an absence or marked decrease in megakaryocytes (fewer than 1 megakaryocyte per 5 high-power fields) within the bone marrow and very little disturbance to the remaining hematopoietic cell lines.[1,10] Because the clinical course of this disorder is quite variable, the only reliable distinguishable characteristic is the lack or marked decrease of megakaryocytes in the face of severe thrombocytopenia. Amegakaryocytic thrombocytopenic purpura can manifest as either a congenital or an acquired syndrome.

Congenital amegakaryocytic thrombocytopenic purpura is a rare disorder of newborn humans that appears to be an inherited defect in megakaryocytopoesis.[4] Given the advanced age of the monkey we present, an inherited syndrome was considered to be highly unlikely.

In addition, AATP is a rare syndrome in the normal human population. Because of the scarcity of AATP, the pathogenesis of this syndrome is not well understood and encompasses a variety of different processes.[11,17] Most commonly, the pathogenesis appears to be an immunologic response by the host that affects the megakaryocyte progenitor line. This response may be cellular

or humoral suppression of the progenitor line or the presence of antimegakaryocyte antibodies or cytotoxic T cells.[7,8] Additional evidence indicates that some cases of AATP are due to an intrinsic defect in megakaryocytic progenitors.[24] Furthermore, other reports describe the possible role of thrombopoietin in this disorder.[24] Thrombopoietin stimulates megakaryocytopoiesis and has been shown to be both increased and decreased in patients with AATP.[24] In general, the evidence indicates that AATP is most likely not a singly defined disorder but rather a culmination of multiple pathologic processes.

In the human literature, potential causes of AATP include drugs, toxins, infectious agents, and immune-mediated disease. In addition, pregnancy can induce pancytopenia with amegakaryocytic thrombocytopenia.[10,11,24] In the presented case, the monkey had not previously received any drugs and, given the controlled housing, ingestion of a toxic substance was considered unlikely.

Viral diseases that have been associated with AATP include measles, dengue fever, and human parvovirus B19.[5,11] Measles is highly contagious in a primate colony and causes considerable morbidity and mortality. However, the presented monkey was vaccinated for measles at weaning, and no outbreaks of measles have occurred within the colony. In addition, clinical signs of measles were absent. Dengue fever has been reported to cause severe thrombocytopenia, with an outbreak in south Texas.[6] Although this monkey had outdoor exposure and mosquito transmission was possible, the likelihood of contracting this disease in the colony's current location (central Texas) was considered to be low. Human parvovirus B19 and parvoviruses in general have been associated with thrombocytopenia, normally in combination with an immunosuppressed state. A single report describes human parvovirus B19 inducing AATP in an immunocompetent but young child (9 mo) that resolved with an infusion of intravenous immunoglobulin.[5] Simian parvovirus was first identified in 1992 and is in the same genus (*Erythrovirus*) as is human parvovirus B19. Simian parvovirus has 50% homology with human parvovirus B19 and very little homology to other parvoviruses.[9] Simian parvovirus has a predilection for erythroid precursors and has been reported to be involved with a decrease in both erythroid and myeloid lineages in the bone marrow of cynomolgus macaques. A review article on simian parvovirus noted that infection is common in macaques.[9,20-22] At our facility, the level of parvovirus exposure is unknown. Because testing for simian parvovirus is not commercially available, the potential for this virus to have factored into the pathogenesis of AATP is unknown.

Pregnancy in humans can be an inciting cause of profound pancytopenia. Most often, pregnancy-induced pancytopenia is linked to aplastic anemia.[19] However, some authors defined a distinct syndrome that is characterized by a cellular bone marrow with reduced or absent megakaryocytes and an elevation of the MCV in peripheral blood. The exact pathogenesis was unknown; however, the syndrome was partially responsive to treatment with immunosuppressive agents, specifically antithymocyte globulin.[19] In our case, the monkey was confirmed pregnant 4 mo prior to the described incident but had a nongravid uterus with vaginal bleeding 2 wk prior to the diagnosis of AATP. Spontaneous abortion does occur at our facility and often goes unrecognized due to the propensity of monkeys to consume both the dead fetus and delivered tissues. Pregnancy with spontaneous abortion cannot be eliminated as the etiology of the current case of AATP. This monkey previously had 4 successful pregnancies with no reported hematologic complications. In humans, AATP associated with pregnancy is reported to occur with the first pregnancy, with subsequent worsening of the condition with following pregnancies. Therefore in our case, symptoms may have been missed in previous pregnancies, with spontaneous resolution of the condition, yet the animal may have been unable to resolve the severity of the pancytopenia in this recent pregnancy.

Immune-mediated diseases such as systemic lupus erythematosus have garnered the most interest as a possible explanation for the lack of megakaryocytopoiesis. In addition, the presence of AATP might indicate early progression to leukemia of various types.[14] A single case report of a patient with AATP noted monoclonal expansion of T lymphocytes that could be an early indicator of a neoplastic process.[7] Other authors have noted AATP with other forms of leukemia, such as large granular lymphocytic leukemia.[16] A related syndrome, immune-mediated thrombocytopenia purpura, causes a marked decrease in platelets due to direct antibody–platelet binding rather than destruction of megakaryocytes. In cases of immune-mediated thrombocytopenia purpura, megakaryocyte numbers in the bone marrow are either normal or increased in both animals and humans.[26,27] Immune-mediated diseases could have played a role in the animal we present, although no other symptoms of immune-mediated disease were noted clinically or on histopathologic evaluation.

Treatment of AATP is as varied as its etiologies and pathogenesis. No therapy is the 'gold standard,' and although resolutions of AATP have occurred, it is unclear which therapeutic approach confers the best prognosis. Immunosuppressive therapies including corticosteroids, cyclosporin A, antithymocyte globulin, danazol, immunoglobulin, cyclophosphamide, or any combination of these pharmaceuticals make up the majority of the therapeutic strategies.[3,13,23-25] Often human patients are misdiagnosed with immune-mediated thrombocytopenia purpura, which often complicates the clinical therapy.

To our knowledge, the current case represents the first published report of AATP in a research-naïve nonhuman primate that is serologically negative for simian retrovirus, SIV, and simian T-lymphotropic virus. The specific cause was not identified definitively; however, the possibility of a recent abortion accompanied by acute onset of vaginal bleeding was considered a possible inciting factor. In addition, investigation into other causes suggested that recent exposure to toxins or infectious agents was unlikely.

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