Successful Treatment of Tacrolimus Related Pure Red Cell Aplasia and Autoimmune Hemolytic Anemia with Rituximab in a Pediatric Cardiac Transplant Patient

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Abbreviations Key

AIHA	autoimmune Hemolytic Anemia
ALT	alanine amino transferase
AST	aspartate aminotransferase
C3d	complement 3d fragment
CMV	cytomegalovirus
DAT	antiglobulin test
dl	deciliter
DNA	deoxyribonucleic acid
EBV	Epstein Barr virus
g	gram

IGG	immunoglobulin G
ITP	immune thrombocytopenic purpura
kg	kilogram
m2	meter squared
mg	miligram
mm3	milimeter cubed
MMF	mycophenolate mofetil
PCR	polymerase chain reaction
PRCA	pure red cell aplasia
RBC	red blood cells
SOT	solid organ transplant
WBC	white blood count

Abstract

Acquired pure red call aplasia (PRCA) and autoimmune hemolytic anemia (AIHA) are rare complications of immunosuppression in pediatric solid organ transplant patients. We report a 14-month-old female who developed Coombs positive hemolytic anemia and reticulocytopenia while on tacrolimus after cardiac transplantation. She was successfully treated with rituximab after failing

treatment with corticosteroids and intravenous immunoglobulins. Clinicians should consider PRCA differential diagnosis in a patient presenting with reticulocytopenia and hemolysis. In addition, the coexistence of PRCA with AIHA, and the response to therapy with rituximab supports a common immune mediated pathogenesis for both disorders.

Introduction

Acquired pure red call aplasia (PRCA) and autoimmune hemolytic anemia (AIHA) are rare complications in pediatric solid organ transplant(SOT) patients with incidence of about 5.6%(1). PRCA is characterized by severe normocytic anemia, reticulocytopenia and erythroblastopenia(1, 2). Congenital forms of PRCA, such as Diamond Blackfan Anemia are caused by genetic defects affecting the erythropoietic lineage(3). Acquired PRCA is associated with malignancy, infections, connective tissue disorders, pregnancy and drugs(1). In SOT recipients, PRCA is most commonly caused by parvovirus B19 infection and immunosuppression with aziathioprine, mycophenolate mofetil(MMF) and tacrolimus being commonly implicated(4). AIHA, on the other hand, is characterized by normocytic anemia, unconjugated hyperbilirubinemia and reticulocytosis caused by increased destruction of red blood cells(RBCs) by anti-RBC autoantibodies with and without complement activation(5).

Although there are reports of either PRCA or AIHA in SOT patients on treatment with tacrolimus, to our knowledge, there are no prior reports of both disorders coexisting in pediatric patients being treated with tacrolimus(6-8). We describe a 14-month-old infant who developed PRCA and AIHA while on post cardiac transplant immunosuppressive therapy with tacrolimus to make clinicians aware of this rare presentation of PRCA and its treatment. We hypothesize that AIHA and PRCA share a common immune mediated pathogenesis and review the literature supporting this hypothesis.

Case Presentation

The patient is a full-term infant with congenital heart block who had a pacemaker placed at 2-days of life. She developed severe cardiogenic shock at age 3-months and was diagnosed with idiopathic cardiomyopathy, CMV hepatitis and hypothyroidism. She was bridged with a ventricular assist device and started on valganciclovir and levothyroxine. At age 7-months she underwent a living donor cardiac transplantation procedure. She was transfused with packed-RBC, platelets, fresh frozen plasma and cryoprecipitate peri-operatively without complications and was started on immunosuppression with MMF and cyclosporine post-operatively. Due to concerns for early rejection, cyclosporine was switched to tacrolimus soon after her first birthday. She presented at 14-months of age with one-week history of worsening lethargy and vomiting. Physical examination revealed severe pallor, but no jaundice, hepatomegaly or splenomegaly. Hemoglobin was 3.2g/dl, reticulocyte $5.8x10^3/mm^3$, and peripheral smear showed normocytic normochromic anemia without significant anisopoikilocytosis[Figure 1A]. WBC was $8.6x10^3/mm^3$, and platelets, $581x10^3/mm^3$. LDH was mildly elevated at 470U/l, haptoglobin low at <10mg/dl, and bilirubin normal. Direct antiglobulin test(DAT) was positive with anti-IgG and C3d specificity and alloantibodies present on adsorption, consistent with immune mediated hemolysis. Liver enzymes were elevated(AST 236U/l and ALT 663U/l). Respiratory viral panel, parvovirus, plasma CMV and EBV DNA PCRs were all negative. She was transfused to a hemoglobin of 7g/dl and discharged home. She became progressively tired and sleepy and was readmitted 5-days later with hemoglobin of 2.9g/dl and reticulocytes of $4.4x10^3/mm^3$. The patient was again transfused and started on a 3-day course of intravenous methylprednisolone(2mg/kg/day) followed by oral prednisone(2mg/kg/day). She

required transfusions every other day to maintain the hemoglobin above 7g/dl even after 3-weeks. A two-day course of intravenous immunoglobulin(1g/kg) was given with neither improvement in reticulocyte count nor transfusion needs. At this point, we suspected a central cause and performed a bone marrow aspirate and biopsy which revealed hypocellularity(50%) with markedly decreased erythroid precursors, consistent with PRCA[Figure 1B and 1C]. She was started on four weekly doses of rituximab(375mg/m^2/dose) with significant improvement in the anemia and reticulocytopenia after the first dose[Figure 2]. One-week after the first infusion, she was no longer transfusion dependent and was discharged home on prednisone taper. Her hemoglobin then was 11.3g/dl and reticulocyte count 299x10^3/mm^3. This reticulocytosis resolved by the 3-month follow-up. Infectious complications included a norovirus infection and cellulitis of the finger both of which resolved on supportive care and antibiotics.

Discussion

This is one of the first reported pediatric cases of concurrent PRCA and AIHA associated with tacrolimus. Due to the rarity of this combined presentation, clinicians may fail to consider PRCA as a possible differential diagnosis in AIHA patients having reticulocytopenia especially as this can be seen in association with normoblastic hyperplasia in 37% of cases of AIHA(9, 10). Reticulocytopenia in these patients is thought to reflect a concomitant immune mediated destruction of RBC precursors, a temporary infection induced suppression of hematopoiesis or a delayed bone marrow response to hemolysis(9). These differ from the typical findings on bone marrow evaluation in PRCA which are complete or near complete absence of erythroid precursors. Although the bone marrow is

usually cellular with unaffected hematopoiesis in the non-erythroid cell lines(1), hypocellularity and a markedly elevated myeloid:erythroid ratio, as in our patient has been described(11) .

PRCA occurring in association with AIHA has been reported in association with hepatitis A, immunosuppressive therapy, lupus(12), lymphoma(13), and Sjogren's syndrome(14) , CMV and EBV infection(15) and in children with no underlying medical problems(16). The interval between onset of PRCA and AIHA varies widely from simultaneous occurrence to 4 years between PRCA and AIHA(2, 17). Post-transplant AIHA and PRCA is generally associated with immunosuppressive therapy. Alemtuzumab, MMF and daclizumab have been implicated(1). Although tacrolimus, another immunosuppressant commonly used in pediatric SOT, has been associated with AIHA or PRCA, the coexistence of both disorders has not been reported to date with tacrolimus.

This case also provides further insight for the possible pathogenesis for PRCA in these patients. Acquired PRCA other than parvovirus B19 are thought to be immune mediated based on the response of these patients to immunosuppression. Soluble serum inhibitors and inhibitory T-cells are believed to inhibit the in vitro growth of and destroy erythroid precursors(3, 16). Support for immune mediated destruction is provided by patients, like ours, in whom PRCA coexists with other known immune disorders like AIHA or ITP. In these patients, the anemia and reticulocytopenia may be due to an accelerated destruction of both erythrocytes and erythroid precursors mediated by a common lineage specific antibody(3, 18). Support for this comes from the isolation of pan-agglutinating auto-antibodies from sera of a group of patients on immunosuppression post SOT(1), a finding comparable to other PRCA pateints in whom, a single antibody directed against both

erythroid precursors and mature RBCs was identified(19). The generation of these antibodies in patients on immunosuppressants, is thought to occur through immune dysregulation(1). Although CMV could be a potential cause of PRCA in this patient, the timing of the PRCA and the absence of significant viremia, which has been described in other cases, makes CMV less likely(15).

The treatment of AIHA involves the use of corticosteroids, IVIG and rituximab in patients who fail first line therapy. Corticosteroids are the first line treatment in patients with PRCA although relapse can occur in 80% of patients during the first year after remission(20). Cyclosporin A may be a better option when long-term maintenance is considered(11). Rituximab has been used successfully in the treatment of PRCA in patients on immunosuppressants(16). Our patient received 4 weekly doses with good response without discontinuing her tacrolimus. Since she was only 7-months post-transplant, it was vital to continue immunosuppression to prevent rejection. Rituximab may also play an additive immunosuppressive role especially in those patients whom humoral mechanisms may be mediating graft rejection.

Clinicians should be aware of this possible coexistence of PRCA and AIHA in patients on treatment with tacrolimus who present with reticulocytopenia and consider performing a bone marrow evaluation in these patients. Rituximab may offer an option for therapy in a selected group of these patients.

Conflict of Interest Statement

The authors do not have any conflict of interests to declare.

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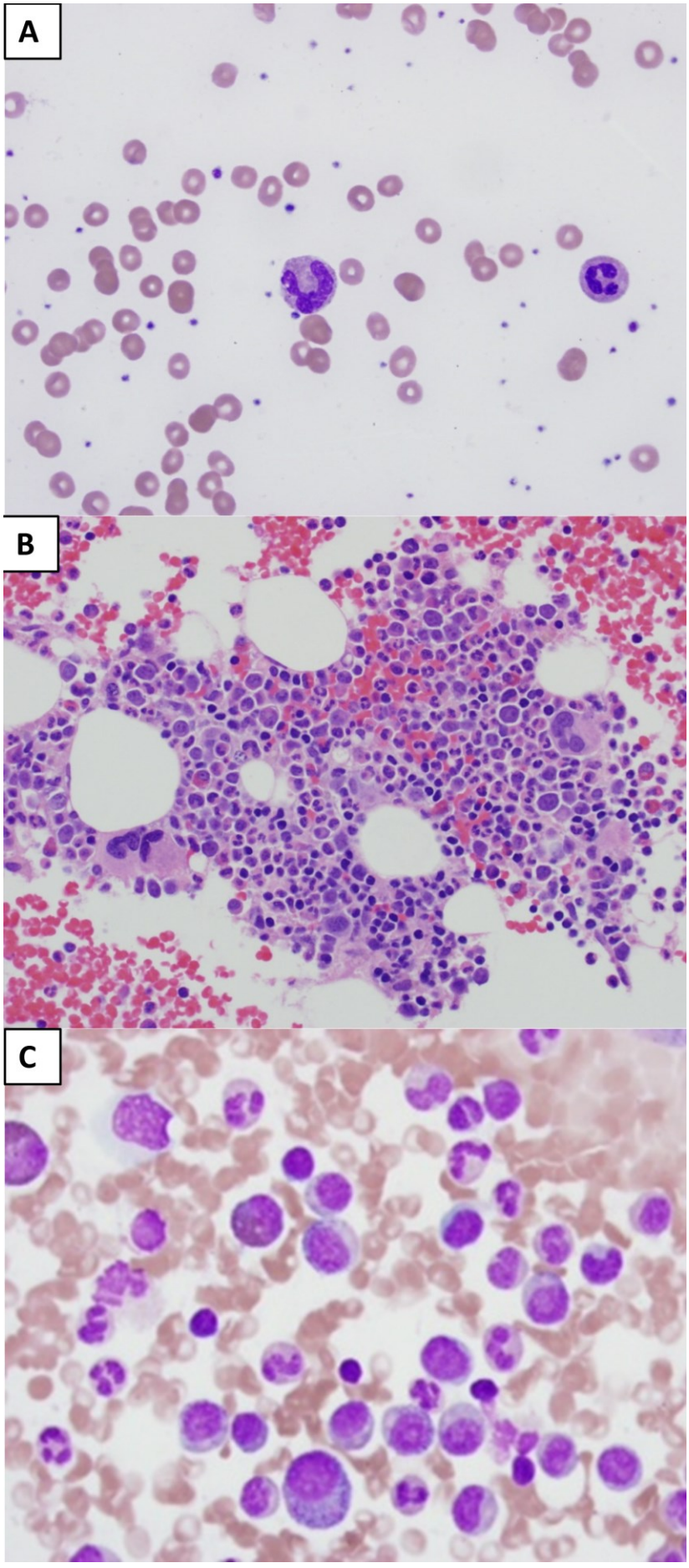
LIST OF FIGURE LEGENDS

Figure 1: The peripheral blood smear normocytic normochromic anemia without significant anisopoikilocytosis [A]. Bone marrow preparations showed a hypocellular bone marrow (50%) cellularity [B and C] with markedly decreased erythroid precursors, normal megakaryocytes and normal granulopoiesis with no increase in blasts.

Figure 2: Hemoglobin and reticulocyte counts showed improvement with stable hemoglobin levels and significant improvement in reticulocyte count following the first dose of rituximab. The transfusion requirements also improved significantly and the patient was off transfusion within the first week following rituximab. Her steroids were also tapered. She remained stable at follow up 3 months after rituximab therapy.



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