Published in final edited form as:

Am J Hematol. 2015 January ; 90(1): 78–81. doi:10.1002/ajh.23791.

Refractory warm IgM-mediated autoimmune hemolytic anemia associated with Churg-Strauss Syndrome responsive to eculizumab and rituximab

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A 29 year-old woman of Filipino descent presented in March 2014 with a several week history of intermittent rash, joint swelling, and shortness of breath. Laboratory tests revealed an eosinophilia (white blood cell count $31.9 \times 10^9/L$, absolute eosinophil count $21.3 \times 10^9/L$), profound anemia (hemoglobin 6.6 g/dL), and mild thrombocytosis. In addition, labs were remarkable for increased hemolysis with elevated LDH, total bilirubin, and undetectable haptoglobin as well as marked reticulocytosis (table 1).

The patient's initial presentation is remarkable for severe peripheral eosinophilia and severe hemolytic anemia in the setting of clinical symptoms of rash and serositis. In general, a single unifying diagnosis is most desired; however, in this setting, a broad consideration of the differential diagnoses for both the eosinophilia and hemolytic anemia is warranted. In addition, further characterization and work-up of both the eosinophilia and hemolytic anemia are needed to narrow the differential. While both issues were investigated in parallel, the work-up for the hemolytic anemia will be discussed first. In general, hemolytic anemia can be classified according to intrinsic RBC defect versus acquired process, usually autoimmune, and intravascular versus extravascular. The initial evaluation includes a careful history of past episodes of hemolysis, evaluation of a peripheral blood smear, and direct Coombs test.

A review of her past medical history revealed no episodes of prior hemolysis. A peripheral blood smear demonstrated full field spherocytes and significant agglutination at room temperature (figure 1A,B). A direct Coombs test was positive and reactive to complement (C3), but was non-reactive to IgG. On further characterization, an IgM autoantibody to red blood cells (RBCs) was suspected based on loss of agglutination upon dithiothreitol (DTT) treatment leading to a suspicion of clinically significant cold agglutinin disease. However, cold agglutinin titers revealed minimal agglutination at 4°C with no elevation in titers.

Author Contributions: MPC, JH, SLS, and RM wrote the manuscript. MPC, JH, SLS, and RM participated in the patient's care. CK and LL provided and analyzed pathology slides.

Thermal amplitude testing revealed agglutination at all temperatures tested; however, titers were progressively elevated from cold to warm temperatures (table 1). Notably the patient did not have any temperature-specific symptoms. To verify the presence of an IgM autoantibody, a “Super Coombs test” (Red Cross Laboratory Los Angeles) was performed, which detected an IgM autoantibody on the patient’s RBCs, but no IgG or IgA autoantibodies. Additional testing revealed no Donath-Landsteiner antibodies, ruling out paroxysmal cold hemoglobinuria, no allogeneic antibodies to minor RBC antigens, and negative testing for paroxysmal nocturnal hemoglobinuria by flow cytometry.

Based on these results, the patient was diagnosed with a warm IgM-mediated autoimmune hemolytic anemia (AIHA). This diagnosis was made due to the finding of an IgM autoantibody detected by Coombs testing, the presence of RBC agglutination seen maximally at room and not cold temperatures, and the exclusion of other causes of agglutination and hemolysis. AIHA due to warm-reacting IgM autoantibodies is exceedingly rare (1). Identification of an IgM autoantibody can be detected by specific anti-IgM antibodies, however, agglutinating IgM antibodies can be present in addition to IgG autoantibodies, which cannot be distinguished in a standard Coombs test. Thus, a common method of detecting an IgM autoantibody is through DDT treatment. DTT inactivates IgM reactivity by reducing the disulfide bonds found in the tertiary structure unique to the pentameric IgM that are absent in other immunoglobulins(2, 3). If an IgM autoantibody is present in the patient serum, DTT treatment will abolish any spontaneous RBC agglutination. To determine that autoantibodies to other immunoglobulins (e.g. IgG and IgA) are not present after DTT treatment, a Super Coombs test can be performed which measures the presence of IgG and IgA antibodies through anti-IgG and anti-IgA reagents. For this patient, DTT treatment abolished RBC agglutination without detection of an additional IgG or IgA autoantibody, demonstrating that an IgM autoantibody was present. There have been only a few case reports of a warm IgM AIHA, mostly associated with immune disorders (4–7). Other reports of warm IgM autoantibodies have been idiopathic in nature (8).

Given the patient’s severe eosinophilia, a parallel workup for primary and secondary causes of peripheral eosinophilia was conducted. The patient did report a prior history of amebiasis in 2004 that was successfully treated without recurrence. She frequently traveled to the Philippines, but had not traveled over the last nine months. Physical examination was notable for hepatomegaly without splenomegaly and a non-blanching vascular patch-like rash on her lower extremities. CT scan of the chest/abdomen/pelvis was remarkable for bilateral pleural effusions and abdominal and pelvic ascites without evidence of lymphadenopathy or masses. Infectious sources causing her eosinophilia were ruled out including negative blood cultures and negative stool cultures for parasites, strongyloides, and toxocara.

The differential diagnosis for peripheral eosinophilia is broad, but generally classified into primary clonal bone marrow disorders or secondary/reactive processes. From a hematologic approach to eosinophilia, reactive causes must first be ruled out before evaluating for primary hematologic causes of eosinophilia. Major reactive causes include infectious, allergic reactions, medication-induced, autoimmune, and malignancy. Infectious etiologies causing eosinophilia mainly include parasitic and helminth infections of which a work up

was negative. The patient did not have evidence of allergic or medication-induced causes. Given the patient's eosinophilia with AIHA, a diagnosis of malignancy was strongly considered. Given the patient's rash, effusions, and symptoms of serositis, an autoimmune cause of her eosinophilia was also considered. Such autoimmune causes include Churg-Strauss Syndrome, systemic lupus erythematosus (SLE), Sjogren's Syndrome, dermatomyositis, and Wegner's granulomatosis. With the exception of SLE, most of these autoimmune disorders are rarely associated with AIHA. Therefore, other etiologies linking both eosinophilia and AIHA were considered, in particular primary bone marrow and hematologic malignancies including Hodgkin's and non-Hodgkin's lymphoma, acute and chronic myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms. Notably, a proportion of patients with peripheral eosinophilia have myeloproliferative neoplasms associated with recurrent genetic abnormalities involving PDGFRA, PDGRB, and FGFR1 (9). The most frequent abnormality in these patients is a FIP1L1-PDGFRA fusion, identified in 23% of patients with hypereosinophilia across multiple case series (9). For this patient, Hodgkin's and non-Hodgkin's lymphoma were unlikely given the lack of lymphadenopathy on clinical exam or by CT imaging. Nevertheless, a bone marrow biopsy is warranted in this case to evaluate for other hematologic malignancies and eosinophilic syndromes.

A bone marrow biopsy was performed which demonstrated hypercellularity with erythroid and eosinophilic hyperplasia, and an absence of abnormal lymphoid infiltrate, dysplasia, blasts, or other malignant populations. Fluorescence in situ hybridization testing for molecular abnormalities associated with primary hypereosinophilic syndromes was negative (table 1). A skin biopsy of the plaque-like rash on the patient's left thigh was performed demonstrating small vessel vasculitis predominantly composed of eosinophils (figure 1E,F). Based on the combination of peripheral eosinophilia, eosinophilic small vessel vasculitis, serositis, and noted prior history of asthma and rhinitis, the patient was diagnosed with eosinophilic granulomatosis with polyangiitis (EGPA), also known as Churg-Strauss Syndrome.

EGPA is an autoimmune vasculitis of the small and medium-sized vessels, usually with multi-organ involvement. The diagnosis of EGPA is based on six clinical criteria developed by the American College of Rheumatology in patients with documented vasculitis, where the presence of four or more had a specificity of greater than 99% (10). These include the presence of asthma, greater than 10% peripheral eosinophilia, mononeuropathy, pulmonary opacities, sinus abnormalities, and biopsy with eosinophilia in extravascular areas. Our patient met four of these criteria, and she was diagnosed with EGPA/Churg-Strauss Syndrome. With the cause of the eosinophilia established, the warm IgM AIHA was presumed to be secondary to the EGPA given the extensive work-up ruling out other causes of AIHA. The primary therapy for EGPA involves systemic steroids and additional immunosuppressive therapy. A comprehensive review of treatment strategies is provided elsewhere (11).

Upon admission, the patient was given an empiric diagnosis of EGPA with AIHA and was initially treated with steroids (methylprednisolone 50mg intravenous daily) for 5 days without improvement in her hemoglobin or indicators of hemolysis. On day 2, she was

transfused 3 units of PRBCs due to her hemoglobin dropping further below 6.6 g/dL. Her anemia initially responded to transfusion, but her hemolysis persisted and her hemoglobin trended down over the next few days while the diagnostic work-up revealed warm IgM AIHA. On day 5, the diagnosis of EGPA was formally made from the skin biopsy and the patient was treated with one dose of cyclophosphamide 700mg/m² IV and three days of high dose methylprednisolone (1g daily). Despite this treatment and the transfusions, the patient's hemoglobin decreased to 5–6 g/dL (figure 2). Given the continued hemolysis, the patient was started on daily plasma exchange to remove circulating IgM autoantibodies in the hope that the immunosuppression would quickly take effect. However, with daily plasma exchange and another round of transfusions, the patient's hemoglobin only transiently increased from roughly 6 g/dL to 8 g/dL with the increase only lasting several hours (figure 2).

The management of this patient's refractory warm IgM AIHA is quite challenging. Warm IgM AIHA carries a poor prognosis including a high incidence of death (1). This is due in part to the lack of efficacy of conventional immunosuppressive therapy that is used in IgG-mediated AIHA. Indeed, our patient received high dose steroids and cyclophosphamide and continued to have active hemolysis. In this case, a short-term immediate therapy was needed to halt the active hemolysis and to serve as a bridge for additional immunosuppression to treat the underlying aberrant B cell-mediated autoantibody production. We expected that plasma exchange would serve as this short-term bridge, however, despite daily plasma exchange, the patient continued to have aggressive hemolysis. Therefore, another therapy that could provide immediate impact on reversing the hemolysis was needed.

We determined that plasma exchange was not likely to lead to a sustained clinical response and opted to treat her with eculizumab, a humanized monoclonal antibody against terminal complement (C5). This decision was based on the finding of complement fixation on the surface of RBCs in the direct Coombs test, as well as evidence of elevated intravascular hemoglobin as indicated by positive urinary hemosiderin. She was administered eculizumab at a dose of 600mg IV on day 9 of admission. Eculizumab was administered after plasma exchange was discontinued to avoid removal of the drug by apheresis. A single dose of eculizumab was administered during this patient's clinical course. Post-administration of eculizumab, the patient's hemoglobin improved significantly and stabilized to 9 g/dL without further need for PRBC transfusions or plasma exchange over a three-day period. Eculizumab was proposed to act by preventing hemolysis once the IgM autoantibody bound to RBCs, but would not be expected to have an effect on autoantibody production itself. In order to further decrease production of the IgM autoantibody, additional immunosuppressive therapy with rituximab, a monoclonal antibody targeting CD20+ B cells, was administered on day 12 at an IV dose of 375 mg/m². The patient's hemoglobin continued to improve and she was discharged several days later on a slow prednisone taper. In the outpatient setting, her hemoglobin continued to improve to 12 g/dL with no recurrence of acute hemolysis. The patient was continued on rituximab 375mg/m² IV weekly for a total of four doses and monthly cyclophosphamide for her EGPA, with her hemoglobin increasing to normal levels with complete resolution of symptoms.

Discussion

Here, we present the case of a 29 year-old woman with newly diagnosed EGPA with an associated warm IgM AIHA that was refractory to high dose steroids, cyclophosphamide, and plasma exchange, but was ultimately responsive to eculizumab and rituximab.

AIHA due to a warm-reacting IgM autoantibody is exceedingly rare (12). While there are no reports of association with EGPA, there have been reports of warm IgM autoantibodies associated with immune disorders including Sjogren's syndrome (4), severe combined immunodeficiency (5, 6), and immune thrombocytopenic purpura (7). Other reports of warm IgM autoantibodies have been idiopathic in nature (8). To our knowledge, this is the first report of a warm IgM AIHA associated with EGPA. AIHA due to warm IgM autoantibodies carries an extremely poor prognosis and is generally refractory to conventional therapies that are effective for IgG-mediated hemolytic anemia (1, 13). Frontline therapies including steroids, IVIg, and splenectomy have not been effective in the few reported cases (6, 13). This is likely due to distinct mechanisms involved in the pathogenesis of warm IgG and IgM-mediated AIHA. Case reports in refractory IgM-mediated AIHA have demonstrated effective use of rituximab (5), interferon alpha 2b (8), and bortezomib (14).

Our patient was refractory to high dose steroids, cyclophosphamide, and plasma exchange. Splenectomy is standard therapy for steroid-refractory IgG-mediated AIHA. This is based on the finding that RBCs bound by IgG are recognized and cleared by macrophages in the spleen; therefore, splenectomy removes the site of RBC clearance and active hemolysis. However, RBCs in IgM-mediated AIHA undergo activation of complement and are not primarily cleared by macrophages in the spleen, but rather likely through ligation of complement receptors expressed on Kupffer cells in the liver. Thus, the liver rather than the spleen is thought to be the site of RBC clearance in IgM AIHA. Consistent with this, the patient did not have splenomegaly but did have hepatomegaly. Our patient exhibited continued hemolysis necessitating a more effective short-term therapy to decrease RBC loss until further immunosuppressive therapy aimed at inhibiting IgM-secreting B cell production could take effect. Plasma exchange was employed to accomplish this short-term goal, but proved ineffective. Therefore, we hypothesized that eculizumab, a monoclonal antibody that targets and inhibits terminal complement protein C5, could be effective in reducing the severe hemolysis in this patient.

Eculizumab is currently FDA approved for the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS); however, use of eculizumab beyond these two indications is limited. Nevertheless, eculizumab has been utilized in two case reports for the treatment of refractory IgM-mediated cold agglutinin disease (15, 16). Indeed, after administration of one dose of eculizumab, our patient's hemoglobin improved from 6 g/dL to 9 g/dL without further drop in hemoglobin or need for transfusions. The patient's LDH and total bilirubin also improved by greater than 50%, supporting the idea that eculizumab caused a reduction in active hemolysis (not shown). This single dose allowed a sufficient bridge to administration of an additional B cell-depleting therapy (rituximab), which led to sustained improvement of the patient's hemoglobin and reduction in hemolysis. This single dose of eculizumab

administered is in contrast to the standard initial weekly dosing used for PNH and aHUS. To our knowledge, this is the first published report of the use of eculizumab for the treatment of a warm IgM AIHA. We speculate that complement inhibition may be an effective therapeutic modality, and that eculizumab may have benefit in warm IgM AIHA as a short-term therapy to improve refractory hemolysis. In addition, rituximab may be an effective long-term therapy for warm IgM AIHA.

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Figure 1. Severe autoimmune hemolytic anemia associated with eosinophilic granulomatosis with polyangiitis

A, B. Peripheral blood smear is shown at low and high power magnification demonstrating eosinophilia, significant RBC agglutination at room temperature (A), and predominant spherocytes (B). Bone marrow core biopsy demonstrates hypercellularity (C) with eosinophilic and erythroid hyperplasia (D). E, F. Left thigh skin biopsy demonstrates eosinophilic infiltration in a small vessel distribution*.

Figure 2. Warm IgM AIHA responsive to eculizumab and rituximab

Hemoglobin is shown during the patient's hospital and outpatient course. Treatments are indicated with doses included below.

Cyclophosphamide: 700mg/m², Eculizumab: 600mg IV, methylprednisolone: 40mg IV, methylprednisolone (high) (1000mg IV), PRBC: 3 units, prednisone 1mg/kg, rituximab: 375mg/m²

PLEX: plasma exchange; PRBC: packed red blood cells; RTX: rituximab

Table 1**Hematology Laboratory Findings**

Laboratory	Result
WBC	3.19×10 ⁴ /μl
Absolute eosinophil count	2.13×10 ⁴ /μl
Hemoglobin	6.6g/dL
MCV	121.4 fL
Platelets	4.65×10 ⁵ /μl
Absolute reticulocyte count	2.9×10 ⁵ /μl
Total bilirubin	1.9mg/dL
LDH	851U/L
Haptoglobin	Undetectable
Coombs test	IgG–, C3+, IgM
Agglutinin titers/thermal	Pan-reactive at all temperatures
Amplitude	4°C 1:8, 22°C 1:16 30°C 1:64, 37°C 1:128
Donath-Landsteiner antibody	Negative for IgG/IgM
Allogeneic RBC antibody screen	Negative
SPIE	Polyclonal
UPIE	Negative
Urine hemosiderin	Positive
PNH flow cytometry	Negative
BCR-ABL translocation (BM)	Negative
JAK2V617F mutation (PB)	Negative
FIP1L1-PDGFRα fusion (BM)	Negative
FGFR1 rearrangement (BM)	Negative
PDGFRB rearrangement (BM)	Negative
T cell clone (BM)	Positive, TCR gamma rearrangement

Laboratory findings are shown for the patient on admission where applicable. BM: bone marrow; LDH: lactate dehydrogenase; MCV: mean corpuscular volume; PNH: paroxysmal nocturnal hemoglobinuria; SPIE: serum protein immunofixation electrophoresis, UPIE: urine protein immunofixation electrophoresis