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Resolution of a steroid-resistant, hypereosinophilic immune diathesis with mepolizumab and concomitant amelioration of a mixed thrombotic microangiopathy

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A B S T R A C T

The anaphylatoxins produced by an unbridled complement cascade in atypical hemolytic uremic syndrome (aHUS) can alter the leukocyte environment in tissues and peripheral blood, causing clinically significant eosinophilia. While the membrane attack complex and C5a anaphlatoxin can be suppressed with anti-C5 biologics, the production of C3a is still capable of driving a destructive hypereosinophilic syndrome in spite of anticomplement therapy. The side-effects of glucocorticoids in treating hypereosinophilic syndrome limit their therapeutic index in long-term treatment and this behooves the use of alternative strategies. While use of the anti-IL-5 antibody, mepolizumab, has been reported for treatment of primary hypereosinophilic syndromes off-label, its usage in the setting of complement-driven thrombotic microangiopathy has not been attempted. We report mepolizumab's rapid resolution of a glucocorticoid-resistant hypereosinophilic syndrome that caused multi-organ dysfunction in a patient with a complex immune diathesis. The patient's long standing TTP/aHUS disease activity, shown to have direct correlation with his eosinophil count, improved with anti-IL-5 therapy, suggesting a reciprocal enhancement between the conditions.

1. Introduction

The eosinophil is a myeloid-lineage-derived granulocyte that is a modulator of both innate and adaptive immunity, as well as an inflammatory effector. Though evolutionarily understood to play an important role in defense against parasites, the eosinophil is recognized in contemporary medicine for its ignoble roles in allergy, atopy, and autoimmunity [1,2].

The role of the eosinophil in immunity can be understood through its modes of participation. Specific granules, containing major eosinophil basic protein, eosinophil peroxidase, eosinophil cationic protein, and eosinophil derived neurotoxin, are the cell's main weaponry. These compounds are capable of inducing oxidative apoptosis and necrosis, mast cell and basophil degranulation, and neutrophil and macrophage activation. Primary granules, containing myeloperoxidase, phospholipase A2, elastase, hydrolases, serine proteases, permeability-increasing-protein, and lysozyme make the eosinophil an able infiltrator, capable of chewing through most tissues to populate the parenchyma [3].

Interestingly, many of the proteins in eosinophil granules have been found to be prothrombotic, and as such, are capable of exacerbating aHUS's feed-forward destruction. For instance, eosinophil peroxidase and major basic protein are platelet agonists and can bind and negate the effects of basophil-produced heparins [4]. Furthermore, major basic protein binds and stymies thrombomodulin [5–7]. Eosinophil peroxidase promotes tissue factor expression [8], while eosinophil cationic protein also plays a minor prothrombotic role in enhancing the activity of factor XII [9]. How clinically meaningful these effects are for the severity of aHUS has not been explored.

Defined as a persistently elevated peripheral absolute eosinophil count above 600 cells/μL for more than six months, eosinophilia spans the gamut of clinical significance from simple urticaria to devastating organ infiltrates. When end-organ damage occurs, or when the eosinophil count reaches 1500 cells/μL in the absence of a parasite or drug reaction, eosinophilia is classified as a hypereosinophilic syndrome. Churg-Strauss disease, now called allergic granulomatosis with polyangitis, is one such entity within this spectrum of these diseases, with many variants both within and outside its diagnostic criteria. The origins of hypereosinophilia are protean. A reactive-based trigger such as one related to an atopic diathesis is most common. However, etiologies related to an underlying hematologic dyscrasia ranging from eosinophilic leukemia, often with translocations PDGFR A and B or FGFR1 [10], to secondary lymphoid-driven etiologies that stimulate

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eosinophil production with soluble factors, most notably interleukin 5 [11,12], can also be observed. In cases where there is no defined trigger, the designation of idiopathic hypereosinophilic syndrome is given. Regardless of cause, whether cycling through the peripheral blood or burrowing into organs, eosinophils are born, sustained, and weaponized by IL-5, and as such, are vulnerable to its removal.

While eosinophils receive development signals from Fc, complement, and anaphylatoxin receptors (such as C3a discussed above), and many cytokines from GM-CSF to TNF-a, IL-5 is a sine qua non for expansion of the eosinophil population [13]. Peripheral counts fall precipitously to near zero levels without the cytokine in mouse KO models [14,15], and anti-IL-5 antibodies drastically reduced the number of eosinophils within tissues and in circulation [16,17]. As a counter-illustration, over production of IL-5 has been shown experimentally to lead to eosinophilia, colon inflammation and autoantibody production [18–20]. Production of IL-5 is limited to Th2 helper T-cells, mast cells, and eosinophils themselves, with IgE complexes, *M. tuberculosis*, and *Toxocara canis* as the ne-plus-ultra of stimulators [21].

IL-5 travels from sites of inflammation to the bone marrow to orchestrate the production and migration of eosinophils. Mature eosinophils leave the bone marrow for 8 to 18 h of circulation and tissue invasion, depending on survival signals [22]. The majority of eosinophils localize to the tissues, particularly at gastrointestinal mucosal surfaces and at sites of Th2-dominated inflammation. Survival of eosinophils in the tissues is enhanced by IL-3, IL-5, GM-CSF, IL-33, and interferon-γ [23].

Anaphylatoxins promote eosinophil extravasation and play a role in shaping in the vascular microenvironment [24]. In the case of complement-mediated tissue pathologies, especially in cases where the disease is being treated with anti-C5 antibodies, C3a acts as a selective chemotactic and infiltrating agent, causing a selective eosinophilic infiltrate and expansion with superiority to C5a [25,26]. Anaphylatoxin inhibitors have been shown to reduce eosinophil counts [27].

Unlike production and preservation, eosinophil activation and degranulation signaling are degenerate, with IL-3, IL-5, GM-CSF, CC chemokines, and platelet activating factor all capable of priming the cell. Among the best inducers of degranulation are anaphylatoxins [28].

IL-5's actions apart from the eosinophil are limited; the nurturing of eosinophils is IL-5's only observed obligatory role. The cytokine's other known functions — the stimulation of humoral response, basophil regulation, and B-cell class switching and maturation towards IgA polyclonal production, are redundantly carried out by TGFbeta1, IL-4, IL-6, IL-10, and IL-13 [29,30].

Since IL-5 is so important in the production of eosinophils and is not required outside of that function, it makes for a perfect target for the treatment of hypereosinophilic syndromes [31]. The first commercial anti-IL-5 antibody pharmaceutical, mepolizumab, was developed by GlaxoSmithKline and had been studied for a decade prior to its FDA approval in 2015. Early mouse studies aligned with later clinical trial data to show an almost complete elimination of peripheral eosinophils and a near 50% drop in eosinophil counts within tissue infiltrates. The monoclonal antibody has been marketed since late 2015 with the sole indication of steroid-resistant eosinophilic asthma [17,32,33].

Below, we detail the use of mepolizumab in the treatment of an increasingly steroid-resistant, anaphlatoxin-driven, hypereosinophilic syndrome in a patient with a history of multiple autoimmune diseases including both TTP and aHUS.

2. Patient, clinical course and results

A 31 year-old Caucasian male with a history of rheumatoid arthritis in remission, 5-ASA-exacerbated ulcerative colitis, atopic dermatitis, asthma, vitiligo, alopecia, RS3PE, and TTP/aHUS who had been treated with myriad combination therapies since 2004 including infliximab, rituximab, adalimumab, prednisone, hydromorphone, hydroxyzine, eculizumab, vincristine, bortezomib, abetacept, cyclosporine, mycophenolate mofetil, methotrexate, and IVIG, and was currently on a q21 day regimen of eculizumab, IVIG, and PRN prednisone, presented to neurology with new diplopia on top of longer-standing fatigue, anemia, and atopy. In 2011, he had been hospitalized for a complement-factor-H-E936D-mutation-driven atypical HUS unmasked by a concurrent TTP [34] which was suppressed with eculizumab therapy [35–37]. Between that initial hospitalization and the end of 2015, the patient's TTP/aHUS disease activity had been only moderately controlled, with multiple flares despite regular eculizumab therapy. In the 16 months leading up to his symptomatic eosinophilia, the patient's average platelet count was 127k/μL, with many peaks and troughs.

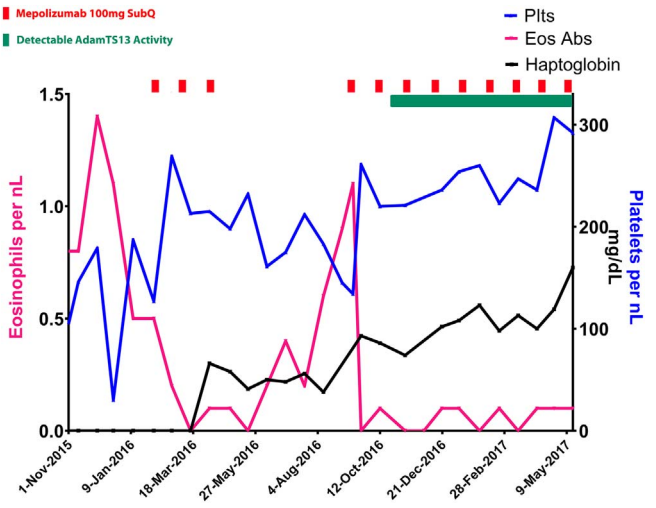

Fig. 1. Recent clinical course coinciding with mepolizumab administration of 100 mg a month.

Peripheral eosinophil counts had been steadily increasing, reaching ~1400 cells/μL in late 2015 (Fig. 1). This prompted a bone marrow biopsy (Fig. 2) by the patient's hematologist with accompanying flow cytometry and cytogenetics. The bone marrow core and aspirate showed a marked eosinophilia, with the aspirate revealing 19% eosinophils (normal 0–3%). Cytogenetics showed a normal karyotype with a FISH panel that was negative for common genetic markers of malignancy. The flow cytometry identified a prominent HLA-DR, CD13, CD33, CD10, CD11b, CD16-positive, CD34, CD117-negative leukocyte population consistent with the phenotypic profile of eosinophils [38]. The same week, and MRI was order by his neurologist and showed three hyperintensities, the largest of which sat in the peri-ventricular splenium of the corpus callosum, with a differential of inflammatory demyelination or CNS lymphoma. Concerned about CNS vasculitis, the patient, who was himself a physician-scientist, sought a specialist at Hospital for Special Surgery and requested mepolizumab to treat the eosinophilia. While insurance hurdles were being confronted, the patient began 60 mg of prednisone every other day in addition to the leukotriene receptor antagonist, monteleukast.

The eosinophil count fell from 1400 to 500 cells/μL, and symptoms of diplopia began to resolve, but not the atopy or colitis. Despite therapy, the eosinophil count remained borderline elevated for the 90 days it took to obtain insurance approval for mepolizumab. Upon injecting mepolizumab on February 16th, steroids and monteleukast were stopped. At three weeks after the first injection, the eosinophil count was 200 cells/μL. The patient's platelet count jumped from 127k/μL to 269k/μL. Two weeks after the second monthly injection, the eosinophil count was 0.0 cells/μL and symptoms of fatigue and atopy had resolved. A second MRI showed shrinking or disappearance of the T2 hyperintensities previously observed. The patient's colitis had also become quiescent. A third dose of mepolizumab was administered on April 5th. After this injection, United Healthcare put mepolizumab on its list of excluded medications and abruptly cancelled coverage of the