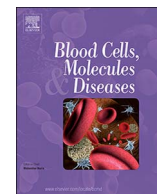




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Resolution of a steroid-resistant, hypereosinophilic immune diathesis with mepolizumab and concomitant amelioration of a mixed thrombotic microangiopathy

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ABSTRACT

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 anaphylatoxin 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 stimulates 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/ μ L 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/ μ L in the absence of a parasite or drug reaction, eosinophilia is classified as a hypereosinophilic syndrome. Churg-Strauss disease, now called allergic granulomatosis with polyangiitis, 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- α , 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 TGF β 1, 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, anaphylatoxin-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, myco-

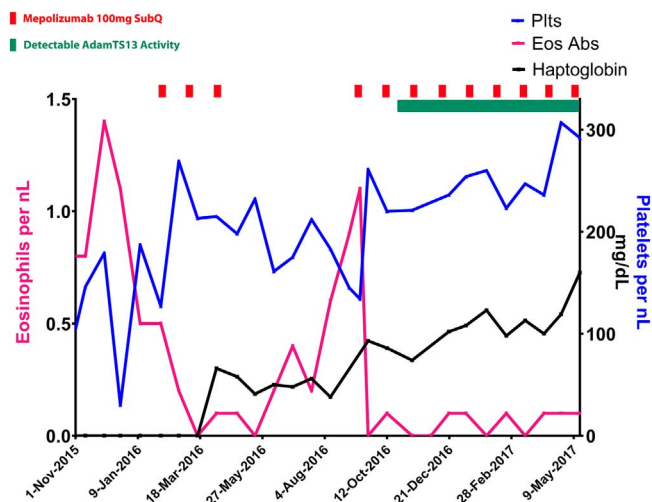


Fig. 1. Recent clinical course coinciding with mepolizumab administration of 100 mg a month.

phenolate 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/ μ L, with many peaks and troughs.

Peripheral eosinophil counts had been steadily increasing, reaching ~1400 cells/ μ L 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, montelukast.

The eosinophil count fell from 1400 to 500 cells/ μ L, 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 montelukast were stopped. At three weeks after the first injection, the eosinophil count was 200 cells/ μ L. The patient's platelet count jumped from 127k/ μ L to 269k/ μ L. Two weeks after the second monthly injection, the eosinophil count was 0.0 cells/ μ L 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

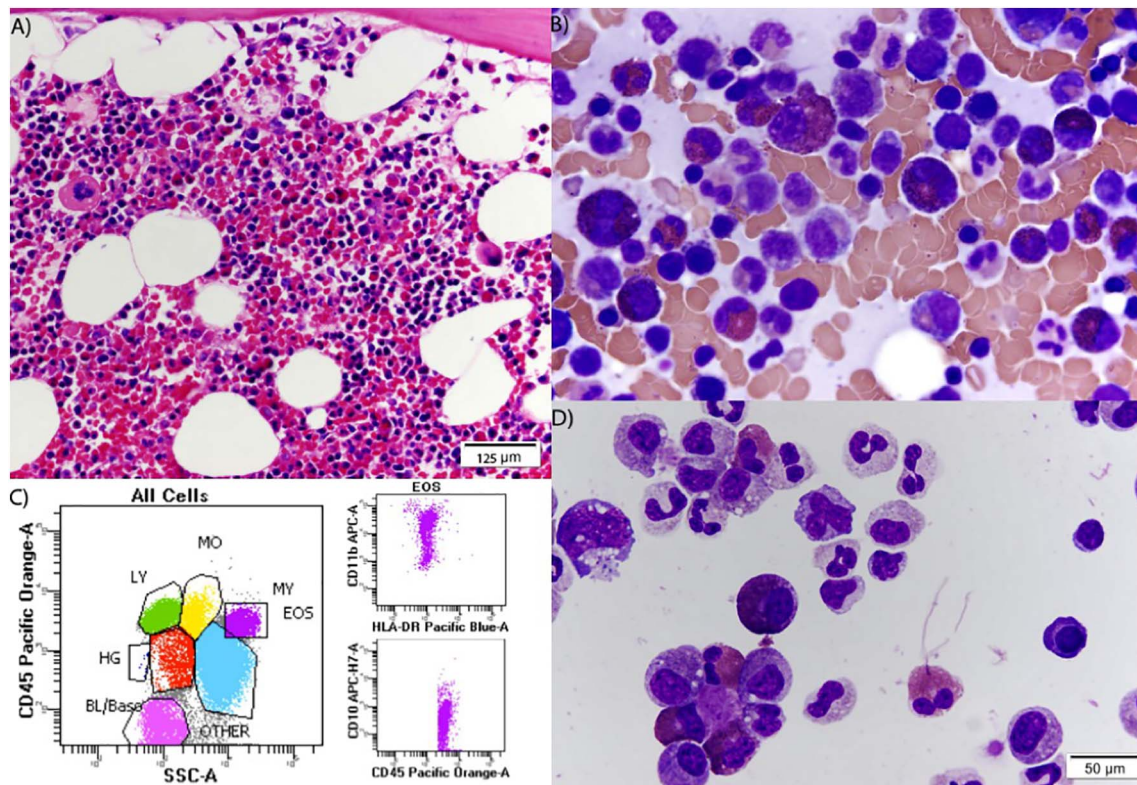


Fig. 2. Full size images available as supplementary material. A) Bone marrow core biopsy showing striking eosinophilia. Hematoxylin and eosin; 40 $\times$ original magnification B) Bone marrow aspirate with eosinophil lineage cells at varied stages of development. Wright-Giemsa; 100 $\times$ original magnification. C) Flow cytometry analysis of cytospin showing an eosinophil population of CD45 and CD11b bright, HLA-DR dim, CD10 negative cells. D) Cytospin analyzed in flow cytometry. Diff-quick; 100 $\times$ original magnification.

drug. The patient was forced to stop mepolizumab therapy.

Upon cessation of mepolizumab therapy, following a short symptomatic reprieve and count trough, the eosinophil count began to sharply increase, and the platelet count dropped. By the end of June, nearly three months after stopping mepolizumab, a steady increase in the patient's eosinophil count to above 1000 cells/ μ L prompted restarting a lower dose of oral steroids and monteleukast. Progressive symptoms of atopic dermatitis, fatigue, diplopia on downward gaze, and now severe to fulminant colitis prompted a return to high dose steroids. Symptoms only slightly improved, and the eosinophil count progression did not abate. On September 13th the patient's new insurance approved the medication. Within three days of mepolizumab injection, the patient was again free of atopic symptoms and colitis. Within nine days his eosinophil count had fallen to 0.00, and his previously undetectable ADAMTS13 activity, a defining feature of TTP, reached its highest point since the beginning of regular testing in 2011. The patient's platelets returned to 261k/ μ L, and have remained within the reference range to date. While unintended and unfortunate, this gap in treatment provided a confirmatory test for the effectiveness of mepolizumab in controlling both signs and symptoms of hypereosinophilic syndrome, and offered a rare opportunity to catalog its relation to aHUS/TTP markers. The patient continues to inject 100 mg of mepolizumab subcutaneously every month.

3. Temporal association of the patient's peripheral blood eosinophilia with the TTP/aHUS course

The patient's eosinophilia first appeared for a span of a few months both numerically and symptomatically in 2007, and then again in 2010, prior to diagnosis of TTP/aHUS. No markers of a thrombotic microangiopathy were assessed during that time. Eosinophil counts later spiked in concordance with a severe mixed thrombotic-macroangiopathic flare in 2011, the medical debut of the patient's complement

dyscrasia. Whether complement was driving eosinophilia prior to the discovery of aHUS is not known. But prior to eculizumab therapy, the full influence of both C5a and C3a anaphylatoxins would have been in effect. Biopsies of the colon during a relatively asymptomatic, fully-treated period in 2014 (Fig. 3) provide clear evidence that eosinophils were still present in tissues that bear heavy breakthrough complement deposition despite regular eculizumab therapy. The colon biopsy in Fig. 3, immunohistochemically stained for c5b-9, showed 3/3 c5b-9 vascular staining in the lamina propria. In contrast, a biopsy of normal skin on the same day showed neither eosinophils nor complement deposition. Thus, even with eculizumab treatment, not just C3a, but to some degree C5a as well, were present in complement staining tissues, capable of enacting eosinophil degranulation and proliferation.

Throughout the patient's clinical course, his hematologists used eosinophils as a marker heralding hemolysis and worsening of TTP/aHUS. Specifically pertaining to this patient, within 48 h following a sudden rise in eosinophil count, predictably, increased creatinine, lower platelets, and a higher LDH were observed. As such, eosinophil spikes were immediately treated with pulse steroid doses to reduce the aHUS flare severity or avert it.

4. Discussion

We have described an unusual case of atypical HUS/TTP overlap with concomitant hypereosinophilic syndrome successfully treated with mepolizumab. The patient experienced a rapid resolution of a glucocorticoid-resistant hypereosinophilic syndrome that caused multi-organ dysfunction. In addition, the patient's long standing TTP/aHUS disease activity, shown to be directly correlated with his eosinophil count, improved with anti-IL-5 therapy, suggesting a reciprocal enhancement between the conditions. This finding highlights the interplay of the cytokine mechanisms underlying his hypereosinophilia and the role of these cytokines in the propagation of his thrombotic microangiopathy.

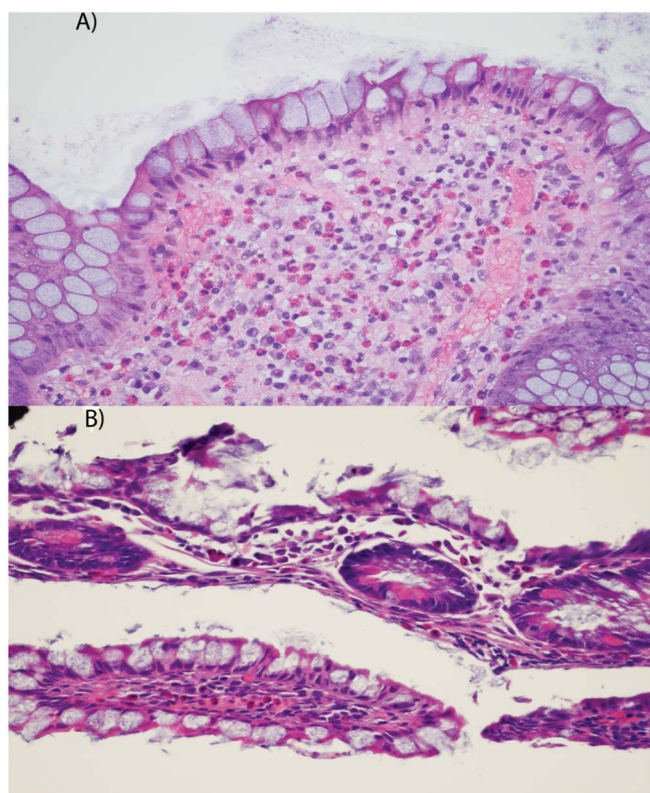


Fig. 3. Representative mucosa from patient of A) Colon and B) Ileum during a relatively quiescent period prior to initiation of mepolizumab administration showing marked infiltration of the lamina propria by eosinophils at 40 × with H & E staining. Pathology service reports that histochemical staining for C5b-9 revealed focally intense 3/3 vascular deposition of complement (original slides not available). No epithelial injury or thrombotic diathesis noted.

The rise in ADAMTS13 activity is one of the more interesting outcomes of his therapy course. It cannot be attributed with certainty to mepolizumab in a patient with extensive polypharmacy, and to date the activity has not increased beyond 57%. The mechanisms of aHUS and TTP are markedly different. To hazard a mechanistic hypothesis, this rise in ADAMTS13 could be related to IL-5's affect on Th2 immune stimulation and a reduction in the strength of humoral immunity.

The nature of the causal relationship between the eosinophilia and aHUS is not readily apparent. Eosinophils express C5a receptor, C3a receptor, complement receptor 3 (CD18) and complement receptor 1 (CD35). Signaling through these receptors is a potent stimulus for respiratory burst. Indeed, since eculizumab halts complement deposition at the C5 stage, full production of C3a and some breakthrough C5a proceeds without the same degree of near-lethal hemolysis. Unlike for neutrophils, which would also be drawn to the region by anaphylatoxins, there is no literature or evidence of eosinophils specifically driving complement fixation; however, they are likely capable of doing so due to the presence of a neutral, serine-like protease, testisin, affixed to their extracellular membrane [39]. In addition, as described above, there is evidence that released eosinophil-granule proteins can drive a prothrombotic state, which could exacerbate thrombotic microangiopathy. Furthermore, in degranulating, eosinophils drawn to the sites of complement activity can further damage endothelium, stimulating even greater complement deposition.

The suppression of eosinophils in the setting of a combined TTP/aHUS was clearly beneficial to this patient, both in relieving the symptoms of hypereosinophilic syndrome and reducing the severity of the thrombotic microangiopathy. Given the nature of aHUS, eosinophilia should not be thought of as a rare occurrence but as a natural and regular outcome of the disease. Accordingly, patients with a complement dyscrasia that has an eosinophilic component may benefit in a

clinically significant way from the addition of mepolizumab to their drug regimen.

A literature search revealed three other cases of TTP complicated by hypereosinophilic syndrome. In these cases, TTP resolved with the reduction of eosinophilia [40–42]. While these cases likely arise from a different mechanism, based on their description of the clinical course, mepolizumab would have likely been of significant benefit. Eosinophilia in the setting of aHUS is more commonly observed [43].

Mepolizumab currently requires reconstitution and injection to administer, and the cost of a month's supply dwarfs that of small molecule DMARDs. While these factors generally relegate mepolizumab to second line therapy, the advantage of an improved therapeutic index and the potential for concurrently ameliorating thrombotic microangiopathies merits consideration of mepolizumab use in both untreated and anti-C5-treated disease.

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