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Changing Roles of Eosinophils in Health and Disease

Glenn T. Furuta, MD^{1,2,3,5}, F. Dan Atkins, MD^{2,4,5}, Nancy A. Lee, PhD^{6,7}, and James J. Lee, PhD^{6,7}

¹Section of Pediatric Gastroenterology, Hepatology and Nutrition, Digestive Health Institute, Children's Hospital Colorado

²Gastrointestinal Eosinophilic Diseases Program, Children's Hospital Colorado

³Mucosal Inflammation Program, University of Colorado School of Medicine, Aurora, CO, USA

⁴Section of Allergy, Immunology, and Rheumatology, Children's Hospital Colorado

⁵Department of Pediatrics; University of Colorado School of Medicine

⁶Department of Biochemistry and Molecular Biology and Division of Hematology and Oncology, Mayo Clinic in Arizona, AZ, USA

⁷Department of Biochemistry and Molecular Biology and Division of Pulmonary Medicine, Mayo Clinic in Arizona, AZ, USA

Introduction

The lack of easily understood activities and/or obvious roles for eosinophils in health and disease have led to a functional ambiguity that is often linked clinically with difficult to treat (and frequently severe) diseases. Interestingly, this functional ambiguity belies a rich history of experimentation and evolving hypotheses that have slowly defined the importance of eosinophils as components of disease processes and the maintenance of homeostasis ¹. In turn, this history of evolving hypotheses has revealed an interesting cyclic pattern of accepted thought that oscillates between descriptions of eosinophils as destructive end-stage effector cells causatively linked with disease pathology or host defense and as anti-inflammatory cells linked with immune modulation, remodeling events, and tissue damage resolution.

An understanding of the historical context surrounding eosinophil biology is relevant as these changing perspectives developed from an underlying need to explain clinical observations and improve patient disease management. As a means of clarity we have divided the history of eosinophils into four significant eras since the formal naming of these cells by Paul Erlich based on their staining properties with the acidic aniline dye eosin ².

I. Paul Erlich to the Mid-20th Century (1880–1960): *Eosinophils are mediators of host defense and causative agents of allergic symptoms and pathologies*

Corresponding Author: James J. Lee, Ph.D., Professor, Mayo Clinic School of Medicine, Department of Biochemistry and Molecular Biology, Director of Special Animal Services Laboratory, Postal Address: Division of Pulmonary Medicine, MCCR-B-RESEARCH; CR-2-213, Mayo Clinic Arizona, 13400 E. Shea Blvd., Scottsdale, AZ85259, Telephone number: (480) 301-7183, FAX Number: (480) 301-7017, jjlee@mayo.edu.

The need to identify and discriminate between cells at the sites of injury and disease was the driving force that led to unique collaborative efforts between late 19th century clinical investigators and the developing chemical/dye industry of the time which led to the creation of staining methods and strategies of cell identification that in many cases survive to present day (reviewed in ³). In regards to the specific identification and initial characterization of eosinophils, Paul Erlich stands out and is generally considered the founding member of the “*Eosinophils ‘H Us’*” club. Even a cursory reading of the literature from this period highlights the accepted perspective of physician-scientists explaining the presence of eosinophils in patients with parasitic diseases and pulmonary subjects with asthma (see for example ^{4, 5}): Eosinophils are innate host defense cells with non-specific destructive activities that target large non-phagocytosable multicellular parasites. Moreover, dysregulated immune responses occurring in the lungs of asthma subjects mistakenly lead to the accumulation of eosinophils in the airways where their non-specific destructive activities result in tissue damage, pathology, and lung dysfunction. Given the limited knowledge of eosinophil activities during this time period and clinical observations with tight correlations between accumulating eosinophils at sites of infection and/or tissues experiencing significant inflammation damage, this hypothesis was plausible. However, it is also a quintessential example of the shortcomings of inductive reasoning – i.e., while the presence of eosinophils correlated with parasite infection and allergic tissue pathologies, no data or clinical studies actually demonstrated a causative relationship.

II. The Early Anti-inflammatory Years (1960–1980): *Eosinophils are recruited to inflamed tissues to dampen the activities mediated by resident pro-inflammatory tissue leukocytes*

Following the definitive identification of eosinophils, a long period of numerous, but nonetheless strictly correlative, clinical studies linking eosinophils with disease pathologies ensued that was also accompanied by a continued inability to define specific eosinophil effector functions. This lack of causality between eosinophil-mediated activities and disease pathology was successfully exploited by investigators who developed hypotheses for the role of eosinophils based on the increasingly specific understanding of effector functions mediated by other leukocytes. This is highlighted by investigators of mast cells who noted that the accumulation of eosinophils appeared to occur in response to the inflammation damage mediated by the activation of accumulating tissue mast cells (reviewed in ⁶). The idea that eosinophil were anti-inflammatory agents naturally grew from the observation that temporally eosinophil accumulation at sites of injury was delayed relative to mast cell activation (e.g., degranulation) and after the initiation of inflammation ⁷. These investigators developed a novel hypothesis that, in the absence of other explanations of eosinophil-mediated activities, became an accepted paradigm: Eosinophils are not destructive effector cells. Instead, they are recruited to sites of pathologies as an anti-inflammatory mechanism(s) to ameliorate the pro-inflammatory activities mediated by activated pro-inflammatory leukocytes such as resident mast cells. As with earlier hypotheses to describe the clinical implications of eosinophil effector functions, this anti-inflammatory paradigm appeared to support what was known given the available studies. However, once again a flaw existed; namely the roles of eosinophils in this paradigm were being defined on the

basis of a greater understanding of the biology of other cells and not on an evolving understanding of eosinophil effector function(s).

III. The “Gleich Era” (1980–2000): *The rebirth of the non-specific and destructive end-stage effector cell hypothesis contributing to both disease symptoms and pathologies*

The advent of molecular biological methodologies including the identification, cloning and characterization of specific genes and the creation of protein-specific single-epitope monoclonal antibodies provided a fulcrum with which the first specific definitions of eosinophil effector functions were possible. The characterization of the genes encoding eosinophil secondary granule proteins (as well as the characterization of the proteins themselves) was spearheaded by Gerald Gleich and several of his contemporaries whose studies dominated this era^{8–21}. The cloning and characterization of these genes revealed an interesting and provocative commonality: the secondary granule proteins were generally very cationic (explaining the propensity of the secondary granules to bind the acidic aniline dye eosin), they possessed in some cases robust enzymatic activities (e.g., ribonuclease and peroxidase activities), and in general were cell cytotoxic (reviewed in⁸). Specifically, upon exposure in cell culture settings^{11, 18} or tissue/organ *ex plant* cultures^{19, 22} the unique enzymatic activities and/or biochemistry that surround these proteins elicited cell death. This phenomenon also extended to multi-cellular parasites that died following exposure to physiologically relevant levels of several eosinophil granule proteins^{16, 17, 23, 24}. Concurrently, clinical studies using immunofluorescence and eosinophil granule protein specific monoclonal antibodies, demonstrated a strong correlation between eosinophil degranulation and evidence of cell death and tissue destruction^{25, 26}. Collectively, the studies of granule proteins and their expression in this era were sufficiently compelling as to shift the accepted paradigm back to where the studies of eosinophils began: Eosinophils through the expression of toxic cationic proteins and other non-specific destructive effector functions (e.g., release of reactive oxygenated species) target parasites as part of innate host defense and the dysregulated accumulation of eosinophils in the airways of asthmatics leads to collateral tissue damage and, in turn, the lung pathology and dysfunction observed in asthma patients.

IV. The LIAR Hypothesis (2000–present): *Eosinophils are critical components of mechanisms necessary for tissue homeostasis through Local Immune And Remodeling/Repair activities*

The advent of clinical studies in asthma patients targeting eosinophils and genetically engineered strains of mice impacting either hypothesized eosinophil effector functions or eosinophils themselves ushered in a new era of eosinophil studies. Initially, animal model studies provided definitive functional assessments of eosinophil-mediated events in the context of *in vivo* settings; asking and answering questions as to the roles of eosinophils in both health and disease. Surprisingly, the earliest of these studies using knockout mice deficient for the eosinophil agonist cytokine IL-5^{27, 28} or IL-5 neutralizing antibodies²⁹ were equivocal regarding the non-specific and destructive end-stage effector cell hypothesis and indeed foreshadowed another changing of perspective that has now become the currently accepted paradigm. For example, although knockout mice deficient for IL-5 on the

background strain C57BL/6J²⁷ led to a concurrent loss of allergen-induced pulmonary eosinophilia and the induced lung dysfunction (airway hyperresponsiveness (AHR)), this was not a universal observation in mice. That is, similar studies on the background strain BALB/c displayed no link between the IL-5 mediated loss of eosinophils and the development of allergen-induced pulmonary pathologies²⁸. The development of biological therapeutics based on these preclinical studies (e.g., monoclonal antibodies specific for IL-5^{30, 31} or the α -chain of the IL-5 receptor³²) displayed equally equivocal results in human subjects. Specifically, clinical studies exploring the use of these reagents interestingly failed to identify direct correlations between eosinophil numbers and pulmonary pathologies with limited effectiveness in only a subset of asthma patients (reviewed in^{33, 34}).

Parallel studies of the potential role(s) of eosinophils using mouse models with complex genetic modifications also failed to support a narrow destructive end-stage effector cell perspective either as agents of host defense or as participants in allergen-mediated respiratory inflammation. These “next generation” genetically-modified mouse models include, knockout mice deficient for either of the abundant cationic secondary granule proteins (MBP-1³⁵ or EPX³⁶) as well as several eosinophil-deficient strains of mice that uniquely target eosinophils in these mice without collateral effects on the production of other cell types^{37–40}. In several studies from multiple groups using multiple strains of mice, little evidence has been reported that supports a significant role for eosinophils in host defense or inflammatory tissue damage. These data include studies directly assessing the roles of eosinophils in host defense against the parasite infection (primary end point measured: worm-burden, using MBP-1 and/or EPX knockout mice^{41, 42} and two different eosinophil-deficient strains (*PHIL* and *dblGATA*) of mice^{43, 44}). Concurrent to these studies were reports using these strains of mice to examine the role(s) of eosinophils as contributors to allergen-induced pulmonary inflammation. In these cases, investigators discovered a similarly confounding result, granule protein knockout mice displayed allergen induced pathology and changes in lung function that were the same as wild type control animals^{35, 36}. The loss of eosinophils entirely also failed consistently to demonstrate a significant eosinophil contribution to cell death/tissue destruction and pulmonary pathology (see for example³⁷ vs.³⁸).

These studies collectively failed to support a perspective in which eosinophils are primarily destructive mediators of host defense and non-specific contributors to localized inflammation. However, a common and underlying observation from these studies was that eosinophils appeared to mediate significant immune regulatory functions in the local areas of interest. This was true of both parasite infection and allergen-induced inflammation. In the case of parasite infection, Appleton and colleagues elegantly showed that while eosinophils did not contribute to *Trichinella* reproduction and/or worm survival, eosinophil modulation of local immune responses in skeletal muscle were absolutely necessary to prevent host inflammatory responses that would otherwise prevent the ability of this pathogen to take up residence at these locations^{44, 45}. Similar observations were noted in studies investigating the roles of eosinophils in allergic respiratory inflammation. That is, instead of destructive end stage cell activities, eosinophil activities in mouse models of

respiratory inflammation were more aptly described as part of pathways modulating immune responses as well as pulmonary remodeling events associated with allergen challenge. These studies included reports of the importance of eosinophil-derived IL-4/IL-13^{46,47}, eosinophil-mediated recruitment of allergen-specific T effector cells to the lung^{48,49}, and eosinophil-dependent effects on T cell proliferation and immune polarization in pulmonary compartment draining lymph nodes⁵⁰. Significantly, very recent studies using both congenitally eosinophil deficient animals and a strain of mice capable of inducible eosinophil-deficiency demonstrated that eosinophils also appear to be part of negative feedback loops that block allergen-induced recruitment/accumulation of airway neutrophils thus shaping the character of induced immune responses/inflammation⁴⁰. Indeed, provocative studies using a wide-range of mouse models of human disease have now demonstrated that eosinophils appear to be key regulators of local immunity and remodeling events linked with diverse tissue settings including adipose tissue homeostasis^{51,52}, liver⁵³ and skeletal muscle regeneration⁵⁴, as well as and neurological diseases (e.g., neuromyelitis optica⁵⁵).

In summary, the preponderance of evidence has led to a partial reversal of the previously accepted perspective and the creation of a larger more inclusive paradigm to explain the roles of eosinophils: Instead of functioning exclusively as end staged effector cells mediating destructive activities as part of innate host defense or dysregulated allergic responses, accumulating eosinophils are also necessary components of local tissue homeostasis by functioning as regulators of *Local Immunity And/or Remodeling/Repair* in both health and disease - The *LIAR Hypothesis*⁵⁶.

Approaches Assessing Eosinophils and/or Eosinophil-mediated Activities in Clinical Settings

Eosinophils have served as the histological hallmark of a number of diseases especially infections, systemic and allergic conditions. For example, in some diseases, such as Churg Strauss Syndrome and hypereosinophilic syndrome, clinical guidelines require a tissue or peripheral eosinophilia, respectively as a diagnostic metric. In other circumstances, eosinophils may also serve as surrogate markers of disease activity as in asthma, atopic dermatitis, allergic rhinitis and conjunctivitis. Finally, an emerging group of diseases termed eosinophilic gastrointestinal diseases (EGIDs) [including eosinophilic esophagitis (EoE), eosinophilic gastritis, eosinophilic gastroenteritis, and eosinophilic colitis] are all characterized by increased eosinophils within the respective tissue spaces. In many of these diseases, therapeutic interventions results in improved symptoms and reduced eosinophilia, findings consistent with disease remission.

While an eosinophil predominance characterizes many diseases, the true impact of these cells in the human condition is not certain. Basic and translational studies have defined clear and distinct roles for eosinophils in patterns of injury such as fibrosis, barrier dysfunction and dysmotility. However, few studies have addressed whether the level of eosinophilia correlates with other features of disease activity. For instance, while standard-of-care practice supports the finding that reduced esophageal eosinophil in EoE occurs following treatment, the degree of symptom improvement as a function of this decreased eosinophilia

is unclear. In fact, several therapeutic trials have shown a significant decrease in mucosal eosinophilia but an inconsistent decline in symptoms. To date, no study has yet determined whether an eosinophil related biomarker can truly serve as a surrogate reflective of symptomatology, natural history, outcome or therapeutic success. Thus, it will be critical for future studies to determine whether quantification of eosinophils and their products should remain a “gold” standard metric.

Methodological tools to assess eosinophilia

Eosinophils can be enumerated in a number of different fashions including counting eosinophils stained with Romanowsky dye sets on peripheral blood smears or stained tissues as well as automated cell counts of liquid samples. Their granule proteins, including eosinophil derived neurotoxin (EDN), eosinophilic cationic protein (ECP), major basic protein (MBP) and eosinophilic peroxidase (EPX) can all be measured by direct assessment by high-throughput detection assays (e.g., ELISA assessments of individual granule proteins and the detection of downstream products of unique eosinophil activities (e.g., detection of brominated and/or nitrated tyrosine residues generated by eosinophil peroxidase-mediated activities). An additional but little used metric is via high throughput mass spec assessments of biological fluid samples (see for example ⁵⁷).

Locations and Samples to assess eosinophilia

Samples to be analyzed are chosen based on their relevance to disease activity. For instance, whereas assessments of peripheral blood and hematological compartments are clearly sites of interest in systemic diseases such as hypereosinophilic syndrome, these compartments are less informative for the assessment of localized inflammatory eosinophil associated diseases, including asthma and EGIDs. That is, while some studies have suggested that peripheral blood eosinophil levels or the peripheral levels of unique eosinophil-specific markers correlate with disease severity ^{58, 59}, the limited power of these studies and the publication of reports with conflicting conclusions ⁶⁰ have limited the usefulness of these systemic evaluations. Instead, the goals of assessment in these diseases have been site-specific sampling. In addition to more targeted tissue assessments of eosinophils in these patients, there is also an underlying move to non- or minimally-invasive methodologies that limit costs and potential sampling complications. In the case of asthma patients, eosinophil assessments in lung tissue from transbronchial biopsies have given way to evaluations of bronchial lavage samples, which in turn, are now giving way to assessments of either induced or spontaneous sputum. Evaluations of EGIDs have also witnessed a similar but still evolving transition of methodologies. That is, endoscopy and colonoscopy with concomitant tissue retrieval were initially the primary methods of assessment for eosinophils that are now giving way to assessments of eosinophil products in stool samples and most recently in luminal fluids. In this regard, methodologies of assessing specific GI compartments in patients are still in development, including cutting edge studies assessing eosinophil-derived products using the minimally invasive EnterotestTM to capture esophageal secretions (i.e., the esophageal string test (EST ⁶¹)).

Successes and Failures of Therapies Targeting Eosinophils in Human Disease

Eosinophil-related disorders vary widely in prevalence, manifestations and morbidity. Moreover, they impact the host in a number of ways depending on whether one organ is primarily involved or the disease is more systemic. For instance, HES affects multiple target organs whereas asthma affects primarily the lungs. Since the pathogenesis of most eosinophil related disorders is unknown, treatments are typically limited to topical or systemic steroids. In some patients with HES the *FIP1L1/PDGFR*A therapeutic target is known, leading to the successful use of imatinib. However, in steroid refractory patients or patients with systemic eosinophilic disorders such as *FIP1L1/PDGFR*A-negative HES or Churg Strauss Syndrome, cytotoxic agents such as interferon- α , cyclophosphamide, hydroxyurea and vincristine are often used^{62, 63}. The potential lack of efficacy, side effects and toxicities associated with these medications require careful monitoring and often complicate patient management. As a result, the search for targeted, safer and more efficacious therapies for eosinophil-related disorders continues.

Interleukin-5 (IL-5)-related targets

IL-5 has long been recognized as a potentially promising therapeutic target given its pivotal role in the terminal differentiation of committed eosinophil precursors and involvement in eosinophil activation, migration and tissue survival⁶⁴. Two humanized anti-IL5 mAbs, mepolizumab and reslizumab, have been developed that bind to IL-5, thereby preventing its interaction with IL-5R α on the eosinophil surface^{64–66}. Another mAb, benralizumab, binds the alpha chain of the IL-5 receptor directly, rendering it unable to bind to IL-5³².

The efficacy of treatment with anti-IL5 or anti-(IL-5) receptor mAbs has been examined in studies of subjects with asthma, EGIDs, atopic dermatitis (AD), CSS, and *FIP1L1/PDGFR*A-negative HES. Interestingly, the results of studies examining the therapeutic effects of these monoclonal antibody treatments in a wide range of diseases have yielded varied outcomes that appear to depend on the patient phenotype and/or the primary endpoint examined. Specific examples of these studies include:

Asthma

Several studies have examined the impact of anti-IL-5 mAbs in the treatment of asthma. Since asthma is a heterogeneous disease, the interpretation of these studies is intrinsically linked to the respective recruited subject populations. For instance, one study revealed that Mepolizumab diminished airway and peripheral eosinophils, but did not impact airway hyperresponsiveness⁶⁷. The results from this study led almost immediately to a diminished interest in eosinophils as a therapeutic target. However, since then a number of studies have provided alternative findings and interpretations. In one randomized, double blind, placebo-controlled study of 24 mildly atopic asthmatics (atopy defined as positive skin prick test to one allergen), Mepolizumab decreased airway mucosal eosinophils and remodeling markers⁶⁸. In two randomized, double blind, placebo controlled, parallel group studies that enrolled 70 asthmatic subjects with mucosal eosinophilia, Mepolizumab led to fewer

exacerbations as well as decreased airway and peripheral eosinophils^{30, 31}. Use of reslizumab has shown similar results in a multicenter trial of 106 asthmatics with sputum eosinophilia and steroid refractory disease. Compared to placebo, reslizumab significantly decreased mucosal eosinophils⁶⁹. In a phase 1 study conducted to determine the impact of anti-IL-5 receptor blockade in asthmatic patients, benralizumab reduced airway mucosal eosinophils and suppressed bone marrow and peripheral eosinophil counts⁷⁰.

Hypereosinophilic Syndrome

Past work demonstrated the ability of Mepolizumab to decrease blood eosinophils by a variety of mechanisms⁷¹. Rothenberg et al conducted an international randomized double-blind, placebo-controlled trial in 85 adults with HES to address the clinical relevance of these studies⁷². They determined that the intravenous administration of Mepolizumab had a clinically significant steroid sparing effect and led to a reduction in peripheral eosinophil counts when compared to placebo.

Eosinophilic GI diseases

A series of case-studies evaluating anti-IL-5 mAb treatment of patients with EGIDs yielded promising results⁷³. Subsequent prospective studies continue to demonstrate a significant impact on the primary endpoint, epithelial eosinophilia. For instance, in a randomized double-blind, placebo-controlled trial of 11 adults with EOE, a significant reduction of mean esophageal eosinophils was observed following Mepolizumab treatment. Additionally, tenascin C and TGF- β 1 expression was significantly diminished⁷⁴. In a larger prospective study of 59 children with EoE, Mepolizumab decreased mucosal eosinophils significantly after 3 infusions separated by 4 weeks each⁷⁵. In the largest EoE therapeutic study to date, 226 adults and children were enrolled in a randomized, double-blind, placebo-controlled study and treated with reslizumab for 12 weeks⁷⁶. Similar to previous studies, peak eosinophil counts declined significantly following treatment. Targeting of IL-5 with mAbs may also diminish inflammation triggered by other cells in EoE including mast cells⁷⁷. Importantly, while there was a trend toward reduction of symptoms in these studies, none documented a significant impact on symptoms when compared to placebo. Potential reasons for this include variability in the symptom score used, delay in resolution of symptom improvement compared to histology, limited dosing, short duration of treatment and lack of penetration of drug into epithelia.

Atopic dermatitis

Because eosinophils are present in atopic dermatitis, anti-(IL-5) antibodies (i.e., MepolizumabTM) have been tested in patients with allergen-induced skin disease. In a randomized double-blind, placebo-controlled study, MepolizumabTM was given intravenously to determine its impact on late phase cutaneous responses⁷⁸. Mepolizumab reduced eosinophil numbers as well as amounts of the remodeling molecule tenascin. A clinical study examined the impact of 2 doses of Mepolizumab in 18 patients compared to 22 controls using the SCORAD tool. No clinical benefit was seen, but peripheral eosinophil counts were decreased⁷⁹.

Taken together, these studies provide evidence that targeting IL-5 or IL-5 receptor with monoclonal antibodies reduces local and/or systemic eosinophils levels. However, a therapeutic benefit for these eosinophil ablations, in most cases, has remained variable. Careful subject selection, dosing approach, including the amount drug delivered, route of administration, and the selection of primary read outs will be critical for the next generation of studies. Future multi-center, randomized, double-blind placebo-controlled studies will also need to be directed at additional therapeutic targets of eosinophils including eotaxins, CTRH2 antagonists, and Th2 associated cytokines ⁶³.

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References

1. Lee, JJ.; Rosenberg, HF., editors. Eosinophils in Health and Disease. Waltham, MA: Elsevier; 2012.
2. Erlich P. Ueber die Specifischen granulationen des Blutes [in German]. Arch Anant Physiol. 1879; 3:571–579.
3. Gleich, GJ. Historical Overview and Perspective on the Role of the Eosinophil in Health and Disease. In: Lee, JJ.; Rosenberg, HF., editors. Eosinophils in Health and Disease. Waltham, MA: Academic Press, Elsevier; 2012. p. 1-11.
4. Dombrowicz D, Capron M. Eosinophils, allergy and parasites. Curr Opin Immunol. 2001; 13(6): 716–720. [PubMed: 11677095]
5. Huber HL, Koessler KK. The Pathology of Bronchial Asthma. Arch Intern Med. 1922; 30(6):689–760.
6. Austen KF. Homeostasis of effector systems which can also be recruited for immunologic reactions. [Review]. J Immunol. 1978; 121(3):793–805. [PubMed: 357653]
7. Durham SR, Craddock CF, Cookson WO, Benson MK. Increases in airway responsiveness to histamine precede allergen-induced late asthmatic responses. J Allergy Clin Immunol. 1988; 82(5 Pt 1):764–770. [PubMed: 3192861]
8. Ackerman SJ, Loegering DA, Venge P, et al. Distinctive cationic proteins of the human eosinophil granule: major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin. J Immunol. 1983; 131(6):2977–2982. [PubMed: 6644025]
9. Peterson CG, Venge P. Purification and characterization of a new cationic protein--eosinophil protein-X (EPX)--from granules of human eosinophils. Immunology. 1983; 50(1):19–26. [PubMed: 6885110]
10. Carlson MG, Peterson CG, Venge P. Human eosinophil peroxidase: purification and characterization. J Immunol. 1985; 134(3):1875–1879. [PubMed: 3918110]
11. Young JD, Peterson CG, Venge P, Cohn ZA. Mechanism of membrane damage mediated by human eosinophil cationic protein. Nature. 1986; 321(6070):613–616. [PubMed: 2423882]
12. Hallgren R, Samuelsson T, Venge P, Modig J. Eosinophil activation in the lung is related to lung damage in adult respiratory distress syndrome. Am Rev Respir Dis. 1987; 135(3):639–642. [PubMed: 3030169]
13. Venge P, Dahl R, Fredens K, Peterson CG. Epithelial injury by human eosinophils. Am Rev Respir Dis. 1988; 138(6 Pt 2):S54–57. [PubMed: 3202523]
14. Gleich GJ, Frigas E, Loegering DA, Wassom DL, Steinmuller D. Cytotoxic properties of the eosinophil major basic protein. J Immunol. 1979; 123(6):2925–2927. [PubMed: 501097]
15. Butterfield JH, Maddox DE, Gleich GJ. The eosinophil leukocyte: maturation and function. Clin Immunol Rev. 1983; 2(2):187–306. [PubMed: 6325060]

16. Hamann KJ, Barker RL, Loegering DA, Gleich GJ. Comparative toxicity of purified human eosinophil granule proteins for newborn larvae of *Trichinella spiralis*. *J Parasitol.* 1987; 73(3): 523–529. [PubMed: 3598802]
17. Molina HA, Kierszenbaum F, Hamann KJ, Gleich GJ. Toxic effects produced or mediated by human eosinophil granule components on *Trypanosoma cruzi*. *Am J Trop Med Hyg.* 1988; 38(2): 327–334. [PubMed: 2451444]
18. Ayars GH, Altman LC, McManus MM, et al. Injurious effect of the eosinophil peroxide-hydrogen peroxide-halide system and major basic protein on human nasal epithelium in vitro. *Am Rev Respir Dis.* 1989; 140(1):125–131. [PubMed: 2751158]
19. Hisamatsu K, Ganbo T, Nakazawa T, et al. Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J Allergy Clin Immunol.* 1990; 86(1):52–63. [PubMed: 2370389]
20. Capron M, Bazin H, Joseph M, Capron A. Evidence for IgE-dependent cytotoxicity by rat eosinophils. *J Immunol.* 1981; 126(5):1764–1768. [PubMed: 7217664]
21. Prin L, Capron M, Tonnel AB, Blettry O, Capron A. Heterogeneity of human peripheral blood eosinophils: variability in cell density and cytotoxic ability in relation to the level and the origin of hypereosinophilia. *Int Arch Allergy Appl Immunol.* 1983; 72(4):336–346. [PubMed: 6417030]
22. Frigas E, Motojima S, Gleich GJ. The eosinophilic injury to the mucosa of the airways in the pathogenesis of bronchial asthma. [Review]. *European Respiratory Journal Supplement.* 1991; 13:123s–135s.
23. Ackerman SJ, Gleich GJ, Loegering DA, Richardson BA, Butterworth AE. Comparative toxicity of purified human eosinophil granule cationic proteins for schistosomula of *Schistosoma mansoni*. *Am J Trop Med Hyg.* 1985; 34(4):735–745. [PubMed: 4025686]
24. Hamann KJ, Gleich GJ, Checkel JL, et al. In vitro killing of microfilariae of *Brugia pahangi* and *Brugia malayi* by eosinophil granule proteins. *J Immunol.* 1990; 144(8):3166–3173. [PubMed: 2324497]
25. Filley WV, Holley KE, Kephart GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet.* 1982; 2(8288):11–16. [PubMed: 6177986]
26. Gleich GJ, Motojima S, Frigas E, et al. The eosinophilic leukocyte and the pathology of fatal bronchial asthma: evidence for pathologic heterogeneity. *J Allergy Clin Immunol.* 1987; 80(3 Pt 2):412–415. [PubMed: 3624694]
27. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model [see comments]. *J Exp Med.* 1996; 183(1):195–201. [PubMed: 8551223]
28. Hogan SP, Matthaei KI, Young JM, et al. A novel T cell-regulated mechanism modulating allergen-induced airways hyperreactivity in BALB/c mice independently of IL-4 and IL-5. *J Immunol.* 1998; 161(3):1501–1509. [PubMed: 9686617]
29. Hamelmann E, Oshiba A, Loader J, et al. Antiinterleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. *Am J Respir Crit Care Med.* 1997; 155(3):819–825. [PubMed: 9117011]
30. Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med.* 2009; 360(10):985–993. [PubMed: 19264687]
31. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med.* 2009; 360(10):973–984. [PubMed: 19264686]
32. Ghazi A, Trikha A, Calhoun WJ. Benralizumab--a humanized mAb to IL-5Ralpha with enhanced antibody-dependent cell-mediated cytotoxicity--a novel approach for the treatment of asthma. *Expert opinion on biological therapy.* 2012; 12(1):113–118. [PubMed: 22136436]
33. Wenzel SE. Eosinophils in asthma--closing the loop or opening the door? *N Engl J Med.* 2009; 360(10):1026–1028. [PubMed: 19264692]
34. Calhoun WJ, Ameredes BT, King TS, et al. Comparison of physician-, biomarker-, and symptom-based strategies for adjustment of inhaled corticosteroid therapy in adults with asthma: the BASALT randomized controlled trial. *JAMA.* 2012; 308(10):987–997. [PubMed: 22968888]

35. Denzler KL, Farmer SC, Crosby JR, et al. Eosinophil major basic protein-1 does not contribute to allergen-induced airway pathologies in mouse models of asthma. *J Immunol.* 2000; 165(10):5509–5517. [PubMed: 11067904]
36. Denzler KL, Borchers MT, Crosby JR, et al. Extensive eosinophil degranulation and peroxidase-mediated oxidation of airway proteins do not occur in a mouse ovalbumin-challenge model of pulmonary inflammation. *J Immunol.* 2001; 167(3):1672–1682. [PubMed: 11466391]
37. Lee JJ, Dimina D, Macias MP, et al. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science.* 2004; 305(5691):1773–1776. [PubMed: 15375267]
38. Humbles AA, Lloyd CM, McMillan SJ, et al. A critical role for eosinophils in allergic airways remodeling. *Science.* 2004; 305(5691):1776–1779. [PubMed: 15375268]
39. Doyle AD, Jacobsen EA, Ochkur SI, et al. Expression of the Secondary Granule Proteins Major Basic Protein (MBP)-1 and Eosinophil Peroxidase (EPX) is Required for Eosinophilopoiesis in Mice. *Blood.* 2013; 122(4):781–790. [PubMed: 23736699]
40. Jacobsen EA, LeSuer WE, Willetts L, et al. Eosinophil Activities Modulate the Immune/Inflammatory Character of Allergic Respiratory Responses in Mice. *Allergy.* 2013 Online ahead of publication.
41. Specht S, Saeftel M, Arndt M, et al. Lack of Eosinophil Peroxidase or Major Basic Protein Impairs Defense against Murine Filarial Infection. *Infect Immun.* 2006; 74(9):5236–5243. [PubMed: 16926417]
42. O'Connell AE, Hess JA, Santiago GA, et al. Major basic protein from eosinophils and myeloperoxidase from neutrophils are required for protective immunity to *Strongyloides stercoralis* in mice. *Infect Immun.* 2011; 79(7):2770–2778. [PubMed: 21482685]
43. Swartz JM, Dyer KD, Cheever AW, et al. *Schistosoma mansoni* infection in eosinophil lineage-ablated mice. *Blood.* 2006; 108(7):2420–2427. [PubMed: 16772607]
44. Fabre V, Beiting DP, Bliss SK, et al. Eosinophil deficiency compromises parasite survival in chronic nematode infection. *J Immunol.* 2009; 182(3):1577–1583. [PubMed: 19155506]
45. Gebreselassie NG, Moorhead AR, Fabre V, et al. Eosinophils preserve parasitic nematode larvae by regulating local immunity. *J Immunol.* 2012; 188(1):417–425. [PubMed: 22131328]
46. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity.* 2004; 20(3):267–277. [PubMed: 15030771]
47. Justice JP, Borchers MT, Lee JJ, et al. Ragweed-induced expression of GATA-3, IL-4, and IL-5 by eosinophils in the lungs of allergic C57BL/6 mice. *Am J Physiol LCMP.* 2002; 282(2):L302–L309.
48. Jacobsen EA, Ochkur SI, Pero RS, et al. Allergic Pulmonary Inflammation in Mice is Dependent on Eosinophil-induced Recruitment of Effector T Cells. *J Exp Med.* 2008; 205(3):699–710. [PubMed: 18316417]
49. Walsh ER, Sahu N, Kearley J, et al. Strain-specific requirement for eosinophils in the recruitment of T cells to the lung during the development of allergic asthma. *J Exp Med.* 2008; 205(6):1285–1292. [PubMed: 18490489]
50. Jacobsen EA, Zellner KR, Colbert D, Lee NA, Lee JJ. Eosinophils regulate dendritic cells and Th2 pulmonary immune responses following allergen provocation. *J Immunol.* 2011; 187(11):6059–6068. [PubMed: 22048766]
51. Wu D, Molofsky AB, Liang HE, et al. Eosinophils Sustain Adipose Alternatively Activated Macrophages Associated with Glucose Homeostasis. *Science.* 2011; 332(6026):243–247. [PubMed: 21436399]
52. Molofsky AB, Nussbaum JC, Liang HE, et al. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med.* 2013
53. Goh YP, Henderson NC, Heredia JE, et al. Eosinophils secrete IL-4 to facilitate liver regeneration. *Proc Natl Acad Sci U S A.* 2013; 110(24):9914–9919. [PubMed: 23716700]
54. Heredia JE, Mukundan L, Chen FM, et al. Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell.* 2013; 153(2):376–388. [PubMed: 23582327]
55. Zhang H, Verkman AS. Eosinophil pathogenicity mechanisms and therapeutics in neuromyelitis optica. *J Clin Invest.* 2013; 123(5):2306–2316. [PubMed: 23563310]

56. Lee JJ, Jacobsen EA, McGarry MP, Schleimer RP, Lee NA. Eosinophils in Health and Disease: The *LIAR* Hypothesis. *Clin Exp Allergy*. 2010; 40(4):563–575. [PubMed: 20447076]
57. Wedes SH, Wu W, Comhair SA, et al. Urinary bromotyrosine measures asthma control and predicts asthma exacerbations in children. *J Pediatr*. 2011; 159(2):248–255. e241. [PubMed: 21392781]
58. Robinson DS, Assoufi B, Durham SR, Kay AB. Eosinophil cationic protein (ECP) and eosinophil protein X (EPX) concentrations in serum and bronchial lavage fluid in asthma. Effect of prednisolone treatment. *Clin Exp Allergy*. 1995; 25(11):1118–1127. [PubMed: 8581845]
59. Stelmach I, Majak P, Grzelewski T, et al. The ECP/Eo count ratio in children with asthma. *J Asthma*. 2004; 41(5):539–546. [PubMed: 15360062]
60. Hui E, Vale RD. In vitro membrane reconstitution of the T-cell receptor proximal signaling network. *Nat Struct Mol Biol*. 2014; 21(2):133–142. [PubMed: 24463463]
61. Furuta GT, Kagalwalla AF, Lee JJ, et al. The oesophageal string test: a novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis and pollen. *Gut*. 2013; 62(10):1395–1405. [PubMed: 22895393]
62. Stone KD, Prussin C. Immunomodulatory therapy of eosinophil-associated gastrointestinal diseases. *Clin Exp Allergy*. 2008; 38(12):1858–1865. [PubMed: 19037962]
63. Wechsler ME, Fulkerson PC, Bochner BS, et al. Novel targeted therapies for eosinophilic disorders. *J Allergy Clin Immunol*. 2012; 130(3):563–571. [PubMed: 22935585]
64. Abonia JP, Putnam PE. Mepolizumab in eosinophilic disorders. *Expert Rev Clin Immunol*. 2011; 7(4):411–417. [PubMed: 21790283]
65. Walsh GM. Profile of reslizumab in eosinophilic disease and its potential in the treatment of poorly controlled eosinophilic asthma. *Biologics: targets & therapy*. 2013; 7:7–11. [PubMed: 23326187]
66. Walsh GM. Mepolizumab and eosinophil-mediated disease. *Curr Med Chem*. 2009; 16(36):4774–4778. [PubMed: 19929788]
67. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet*. 2000; 356(9248):2144–2148. [PubMed: 11191542]
68. Flood-Page P, Menzies-Gow A, Phipps S, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest*. 2003; 112(7):1029–1036. [PubMed: 14523040]
69. Castro M, Mathur S, Hargreave F, et al. Reslizumab for Poorly Controlled, Eosinophilic Asthma. *American Journal of Respiratory and Critical Care Medicine*. 2011; 184(10):1125–1132. [PubMed: 21852542]
70. Laviolette M, Gossage DL, Gauvreau G, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. *J Allergy Clin Immunol*. 2013; 132(5):1086–1096. e1085. [PubMed: 23866823]
71. Menzies-Gow A, Flood-Page P, Sehmi R, et al. Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol*. 2003; 111(4):714–719. [PubMed: 12704348]
72. Rothenberg ME, Klion AD, Roufosse FE, et al. Treatment of patients with the hypereosinophilic syndrome with mepolizumab. *N Engl J Med*. 2008; 358(12):1215–1228. [PubMed: 18344568]
73. Stein ML, Collins MH, Villanueva JM, et al. Anti-IL-5 (mepolizumab) therapy for eosinophilic esophagitis. *J Allergy Clin Immunol*. 2006; 118(6):1312–1319. [PubMed: 17157662]
74. Straumann A, Conus S, Grzonka P, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut*. 2010; 59(1):21–30. [PubMed: 19828470]
75. Assa'ad AH, Gupta SK, Collins MH, et al. An antibody against IL-5 reduces numbers of esophageal intraepithelial eosinophils in children with eosinophilic esophagitis. *Gastroenterology*. 2011; 141(5):1593–1604. [PubMed: 21835135]
76. Spergel JM, Rothenberg ME, Collins MH, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *The*

- Journal of allergy and clinical immunology. 2012; 129(2):456–463. 463 e451–453. [PubMed: 22206777]
77. Otani IM, Anilkumar AA, Newbury RO, et al. Anti-IL-5 therapy reduces mast cell and IL-9 cell numbers in pediatric patients with eosinophilic esophagitis. *J Allergy Clin Immunol*. 2013; 131(6): 1576–1582. [PubMed: 23623266]
78. Phipps S, Flood-Page P, Menzies-Gow A, Ong YE, Kay AB. Intravenous anti-IL-5 monoclonal antibody reduces eosinophils and tenascin deposition in allergen-challenged human atopic skin. *J Invest Dermatol*. 2004; 122(6):1406–1412. [PubMed: 15175031]
79. Oldhoff JM, Darsow U, Werfel T, et al. Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. *Allergy*. 2005; 60(5):693–696. [PubMed: 15813818]