EOSINOPHIL BIOLOGY AND DISORDERS

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Disclosure

- No relevant financial relationships to disclose

- None of the drugs discussed, with the exception of imatinib, are approved for the treatment of hypereosinophilic syndrome (HES). This includes prednisone, hydroxyurea, interferon, alemtuzumab, cyclosporine, mepolizumab, reslizumab, benralizumab, and any other agents discussed.
Learning Objectives

Upon completion of this session, participants should be able to:

• Describe the basic biology of eosinophils
• Recognize the heterogeneity of eosinophilic disorders
• Describe the different subtypes of HES
• Employ a systematic approach to the treatment of HES
Eosinophilia: Case 1

- 28 year old Hispanic man with history of intravenous cocaine use presents with signs and symptoms of severe right-sided congestive heart failure in the setting of marked eosinophilia
  - wbc 14,400 with 63% eosinophils (9,072/mm3), anemia, platelets 90k
  - echocardiogram: dilated cardiomyopathy, fibrotic material filling the right ventricle, moderate to severe MR and TR, small pericardial effusion
  - endomyocardial biopsy: focal fibrosis
  - bone marrow: hypercellular with marked eosinophilia, no blasts, mild fibrosis
Eosinophilia and endomyocardial fibrosis

- Drug hypersensitivity
- Churg-Strauss Syndrome
- Parasitic infection
- Hypereosinophilic Syndrome
Case 1 (continued)

• A diagnosis of hypereosinophilic syndrome was made and he was treated with high dose steroids and hydrea without response

• Approximately 10 months after he presented, imatinib mesylate therapy was started with complete hematologic remission

• One month later he developed hemoptysis, fever and chills and died of presumed sepsis

• Post-mortem diagnosis: \textit{FIP1L1/PDGFRA}-positive MPN
Questions

• What are eosinophils?
• What do eosinophils do?
• My patient has eosinophilia. Now what?
What are eosinophils?

- Eosinophils are terminally differentiated cells of the myeloid lineage that stain red with negatively charged eosin
Eosinophil life cycle

1-2% of peripheral blood leukocytes; t_{1/2} in blood = 18 hours

>90% of eosinophils are found in the tissues, particularly those tissues which interface with the environment (BM, lymphoid tissue, lower GI tract, and uterus)

(from Rothenberg 1998 NEJM)
Normal eosinophil morphology

- Bilobed nucleus
- Vesiculotubular network
- Secondary granules (core and matrix)
- Primary granules (Charcot-Leyden protein)
- Cationic proteins (EDN, MBP, ECP, EPO)
- Enzymes (lysozyme, elastase, cathepsin D, ...)
- Cytokines (IL4, IL5, IL6, ...)
- Lipid bodies (cyclooxygenase, lipoxygenase, leukotrienes, EPO, ...)

Image of eosinophil showing these structures.
Eosinophil activation

**Altered phenotype**

- Density: hypodense
- Light microscopy: dysplasia, cytoplasmic clearing
- EM: piecemeal degranulation, increased lipid bodies
- Surface marker expression: increased CD69, CD44, CD25, HLADR, decreased CD23

**Enhanced functions**

- Prolonged survival
- Adhesion
- ADCC
- Chemotaxis
- Mediator release: ECP, MBP, EDN, LTC4, ROS
- Cytokine production: IL8, GM-CSF, IL5, …
Eosinophil ‘specific’ granules

- Intact granules are released upon cell cytolysis
- Free and intracellular granules can differentially release their contents (piecemeal degranulation)
- Granule and granule protein release has been associated with DNA nets, tissue damage and fibrosis
- Serum levels of granule proteins have been correlated to disease activity in some settings

Neves and Weller, 2009
Questions

• What are eosinophils?
• **What do eosinophils do?**
• My patient has eosinophilia. Now what?
The Eosinophil: Friend or foe?

- Tissue remodeling
- Host defense against helminths
- Tumor surveillance
- MHC class I-restricted thymocyte depletion
- Modulation of the immune response
  - Antigen presentation
  - Cytokine and chemokine secretion
  - Maintenance of long-lived plasma cells
  - Glucose homeostasis
- Allergic inflammation
  - Direct cytotoxic effects
  - Recruitment of other inflammatory cells
- Fibrosis
- Thromboembolism
Eosinophils and homeostasis

Brink Nat Immunol 2011
Chu et al. Nat Immunol 2011

Maizels and Allen Science 2011
Wu et al. Science 2011
What can we learn from eosinophil-free mice?

- Eosinophil-free mice appear normal, but have altered responses to allergen challenge
  - *EPO-DTN* mice (Lee et al. Science 2004)
  - Ddbl GATA (Humbles et al. Science 2004)

- Effect in helminth infection depends on model
  - Schistosomiasis – no effect (Swartz et al. Blood 2006)
  - Trichinella infection - decreased parasite survival (Fabre et al. J Immunol 2009)
Are eosinophils essential?

**FOR**
- They are primitive cells found in all vertebrates (even zebrafish have them)
- Congenital eosinophil deficiency has not been reported in humans

**AGAINST**
- Rare cases of acquired eosinophil deficiency have been reported in humans (summarized in Gleich et al. Allergy 2013)
- Profound depletion of eosinophils in humans using targeted therapies does not appear to have adverse consequences
Questions

- What are eosinophils?
- What do eosinophils do?
- My patient has eosinophilia. Now what?
Clinical disorders associated with eosinophilia

- Allergy/Asthma
- Drug hypersensitivity
- Connective tissue disorders
- Parasitic infection
- Neoplasm
- Rare hypereosinophilic syndromes
- Other (hypoadrenalism, HIV...)
How high is too high?

CAVEATS

- Percent is not useful
- May be suppressed by:
  - bacterial and viral infections
  - fever
  - corticosteroids
- Diurnal variation
- Lab mistakes can occur
- Peripheral blood eosinophil count > 450/μl occurs in 5-10% of returned travelers
- Even mild eosinophilia can be associated with disease, but the differential diagnosis depends on the level
Asthma and eosinophilia

- Common cause of mild to moderate peripheral eosinophilia
- Sputum eosinophilia is also present in a subset of patients
- Dramatic eosinophilic airway inflammation has been demonstrated post-mortem in patients who died of asthma
- Corticosteroids are effective in reducing eosinophilia and in asthma treatment

BUT: early trials of anti-IL5 antibody showed no effect in asthma despite a decrease in blood and tissue eosinophilia
Anti-IL5 therapy is effective in treating some patients with asthma

Mepolizumab and Exacerbations of Refractory Eosinophilic Asthma


Mepolizumab for Prednisone-Dependent Asthma with Sputum Eosinophilia

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Hypereosinophilia and hypereosinophilic syndromes

- Blood eosinophilia $\geq 1500/\mu$L on at least two occasions or evidence of prominent tissue eosinophilia associated with marked blood eosinophilia
- Evidence of end organ damage attributable to eosinophilia

NOTE: Secondary causes of eosinophilia, such as parasitic or viral infection, allergic diseases, drug- or chemical-induced eosinophilia, hypoadrenalism and neoplasms, can cause HES, but need to be identified as they require specific treatment directed at underlying cause

(Klion et al. JACI 2006; Simon et al. JACI 2010; Valent et al JACI 2012)
End Organ Involvement in HES

Ogbogu et al. 2009 JACI
Secondary causes that mimic rare HES

- 50 year old Iraqi rug salesman with pruritus and rash consistent with erythema multiforme, HES, 30,000/mm³
- 32 year old businessman with acute CHF while in Benin, West Africa. CBC showed eos 30,000/mm³, echocardiogram consistent with EMF

![Pie chart showing diagnosis distribution: HES, helminth, drug, neoplasm, other.]

- H and E
- Anti-MBP
Eosinophilia and drug reactions

- Most common cause of HE in US and Europe
- May be provoked by any drug or supplement
- Can be asymptomatic or associated with characteristic signs and symptoms
  - Asymptomatic- quinine, PCNs, cephalosporins, quinolones
  - Pulmonary infiltrates-NSAIDs, sulfas, nitrofurantoin
  - Hepatitis - tetracyclines, semisynthetic PCNs
  - EMS - L-tryptophan contaminant
  - Interstitial nephritis- cephalosporins, semisyn. PCNs
  - Drug rash with eosinophilia and systemic symptoms (DRESS) - anti-epileptics, NSAIDs, antibiotics,…
Eosinophilia and non-parasitic infectious diseases

- Most **acute** bacterial and viral infections are associated with **eosinopenia**
  - Eosinopenia is a poor prognostic sign in sepsis
  - Eosinophilia in a patient with systemic inflammatory response syndrome (SIRS) should prompt investigation for a non-bacterial/viral etiology

- **Eosinophilia** is a feature of some non-parasitic infections
  - Bacteria: resolving scarlet fever, chronic tuberculosis
  - Fungi: coccidiomycosis, allergic bronchopulmonary aspergillosis (APBA) and others
  - Viruses: HIV
Eosinophilia and protozoan infections

- Not characteristic with the exception of *Isospora belli* infection and *Sarcocystis*

(image courtesy of CDC DPDx)
Eosinophilia and ectoparasites

- **Scabies**
  - sensitization to the mites and their eggs causes itching, erythema, rash, and may cause eosinophilia

- **Myiasis**
  - Infestation by fly larvae has rarely been associated with hypereosinophilic syndrome

(images courtesy of CDC DPDx)
Eosinophilia and helminth infection

- Helminth infection is the most common cause of eosinophilia worldwide
- Eosinophilia is most pronounced in the setting of tissue invasion (often early in infection)
- Eosinophilia lasting >3 years is seen in relatively few infections
  - hookworm, flukes (i.e., schistosomiasis, fascioliasis, clonorchiasis), filariasis, gnathostomiasis, strongyloidiasis

(image courtesy of CDC DPDx)
Eosinophilia: case 2

• 23 year old Peace Corps volunteer recently returned from Africa complaining of hives x 3 months
• PE is unremarkable except for skin exam, which shows this lesion on the lower back
• Labs notable for AEC 4,500/μL
What is the most likely diagnosis?

(A) Hypereosinophilic syndrome
(B) Strongyloidiasis
(C) Cutaneous larva migrans
(D) Drug eruption
(E) Scabies
Clinical subtypes of rare HES

NIH COHORT (n=263)

- MYELOPROLIFERATIVE
- LYMPHOCYTIC
- OVERLAP
- ASSOCIATED
- FAMILIAL
- IDIOPATHIC
Elevated serum tryptase identifies a myeloproliferative subtype of HES

- Male gender
- Anemia and/or thrombocytopenia
- Dysplastic eosinophils and myeloid precursors in periphery
- Splenomegaly
- Hypercellular marrow
- Increased serum B12 levels
- **30% mortality at 3 years**

(Klion et al. 2003 Blood)
PDG FRA-associated MPN

- Caused by an interstitial deletion in chromosome 4 that leads to a constitutively activated fusion tyrosine kinase that is sensitive to imatinib (Cools et al. NEJM 2003)

- Can be detected by nested RT-PCR or FISH

- A number of additional fusion partners, as well as point mutations, have now been identified (Gotlib 2004 Blood)
Additional clinical features of PDGFRA-associated MPN

- Extreme male predominance
- Bone marrow mastocytosis and elevated tryptase is usually, but not always seen, but without classic symptoms of mast cell activation (i.e. anaphylaxis, flushing, diarrhea)
- Unusual dermatologic presentations have been reported
  - Mucosal ulcerations
  - Lymphomatoid papulosis (LyP)

(Thurny JAEDV 2010) (McPherson BrJDerm 2008)
Myeloproliferative variant HES

- *PDGFRA*-positive MPN (>80%)
- CEL-NOS
  - Demonstrable cytogenetic abnormalities and/or increased blasts
- Idiopathic HES with myeloproliferative features

NOTE: marked eosinophilia also occurs in myeloid neoplasms and myeloproliferative disorders, including those associated with *FGFR1*, *KIT*, and *JAK2* but the clinical features are due to the involvement of other lineages in most cases
Lymphocytic variant HES

- Associated with populations of phenotypically aberrant or clonal T cells secreting eosinophilopoietic cytokines
- Equally common in men and women
- Predominance of skin manifestations
- Often associated with elevated serum IgE, TARC levels
- May progress to lymphoma
  - <3%; usually preceded by cytogenetic abnormalities and lymphocytosis
- Imatinib is not effective
Production of IL-4 and IL-5, and not IFN-\(\gamma\), by CD3-CD4+ T cells in LHES
Novel variants of LHES

- **EBV-driven LHES**

63 year old man with a 2 year history of ulcerative lesions

[Image: Bright field and FISH-EBV (594) images]

- EBV-transformed B cells (X50-7)
- EBV-negative B cells (BJAB)
- V$\beta$ 5.1 positive patient cells

Pretreatment

EBV copies

(Klion et al. 2013 Blood)
Novel variants of LHES

- Episodic angioedema and eosinophilia (EAE; Gleich’s syndrome)

36 year old man with recurrent episodes of bilateral hand and foot swelling x 3 months

Additional features
- CD3-CD4+ clonal T cell population
- Elevated IgM
- Multilineage involvement

(Katzen Am J Dis Child 1986)
Overlap: Eosinophilic gastrointestinal disorders (EGID)

- Eosinophilic infiltration of the gastrointestinal tract
  - Eosinophilic esophagitis
  - Eosinophilic gastritis
  - Eosinophilic enteritis
  - Eosinophilic colitis
  - Eosinophilic gastroenteritis

- Signs and symptoms vary depending on location of involvement
- May be accompanied by peripheral eosinophilia >1,500/mm$^3$
Overlap: EGPA (aka Churg-Strauss Syndrome)

Churg and Strauss, 1951- Autopsy series
1. Asthma
2. Necrotizing vasculitis of small and medium vessels
3. Eosinophil infiltration around vessels and tissues
4. Extravascular granulomas
5. Fibrinoid necrosis of involved tissues

ACR, 1990- Research definition to distinguish CSS from other forms of vasculitis
1. Asthma
2. Eosinophilia >10%
3. Neuropathy
4. Pulmonary infiltrates
5. Paranasal sinus abnormality
6. Extravascular eosinophil infiltration on biopsy
Idiopathic HES

- Despite extensive evaluation, in >50% of patients with eosinophilia >1500/μL, no etiology is apparent and the clinical picture does not fit one of the defined subtypes.

- A subset of these patients are completely asymptomatic and have no evidence of end organ involvement (hypereosinophilia of unknown significance; HEUS). (Chen JACI 2013)
Conventional therapy for HES

Prednisone
Hydroxyurea
Interferon-α
Imatinib (FDA-approved for HES in 2006)

Response at 1 month

(Ogbogu et al 2009 JACI)
Second line therapy for HES

- *PDGFRA*-positive MPN: second generation TKI or BMT
- *PDGFRA*-negative myeloproliferative HES: imatinib
- Lymphocytic variant HES: interferon-alpha
- Idiopathic HES: interferon-alpha or hydroxyurea
- HES with features of EGPA: methotrexate

What next?
Targeted therapies on the horizon

(Bochner JACI 2012)