The Eosinophil

Basic & Clinical Aspects

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Disclosures

- Grants through Cephalon for investigation of eosinophil participation in disease
- Consultant to GSK and Cephalon
- Royalties from Cephalon

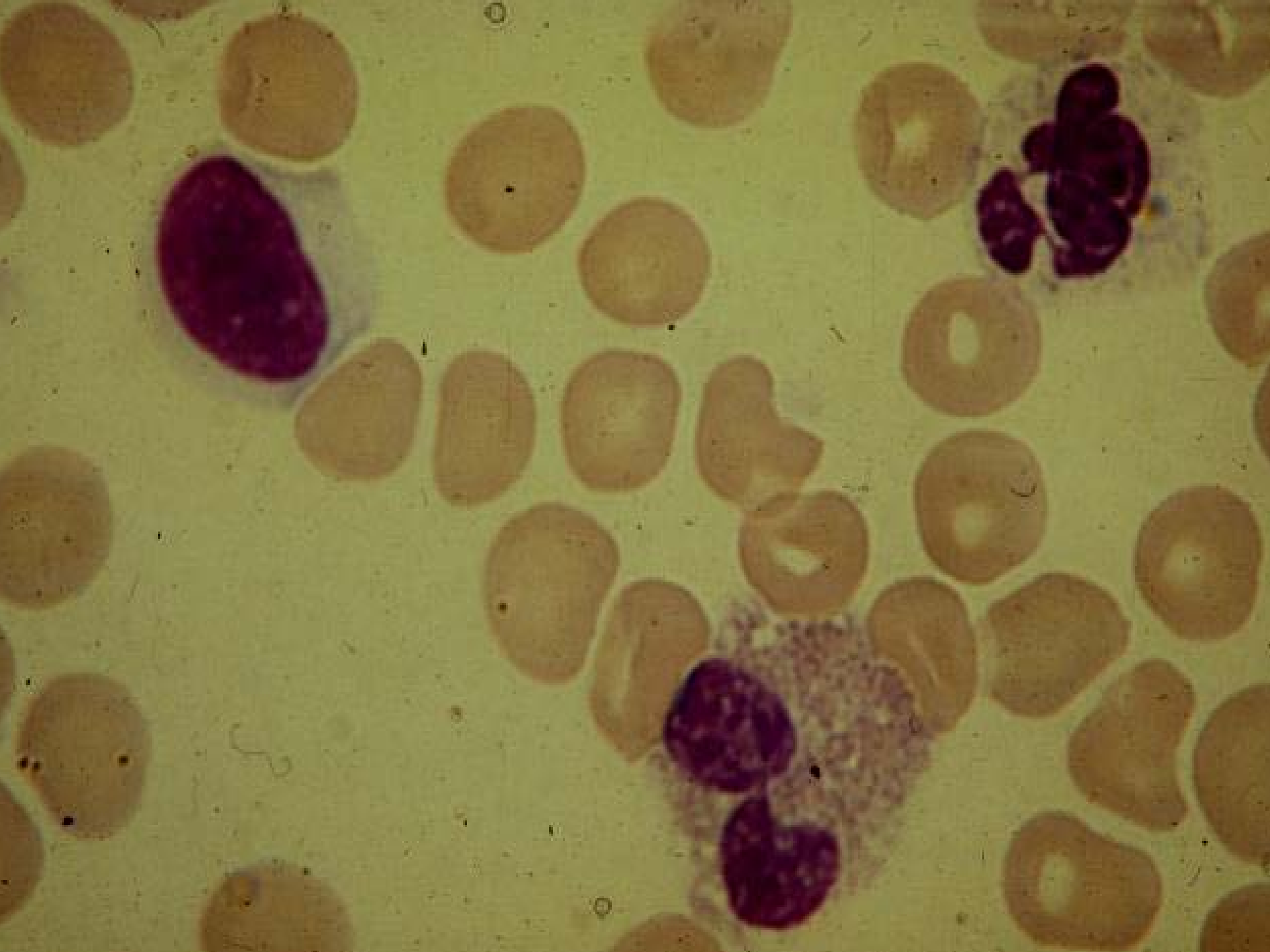
The Eosinophil

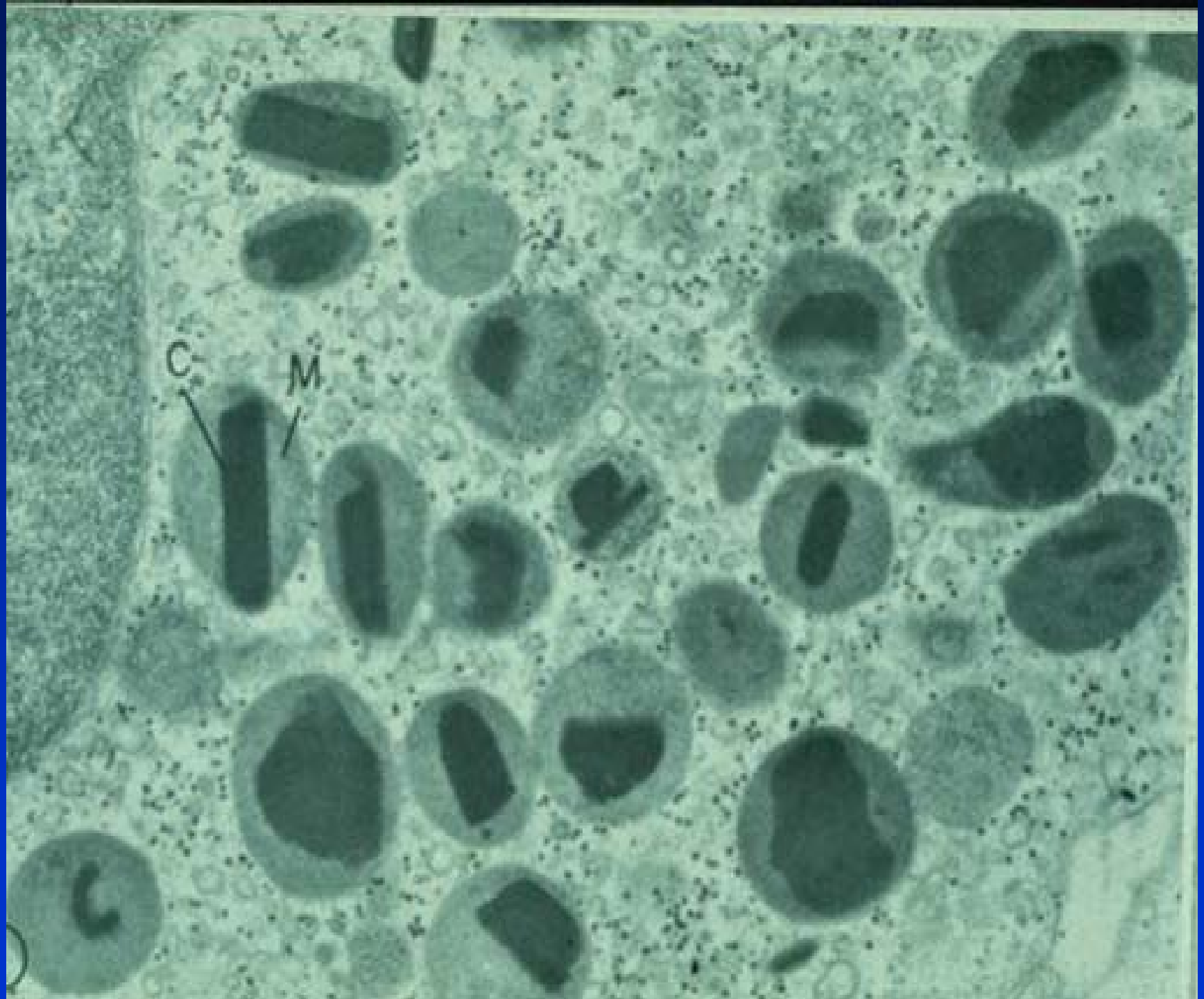
Basic & Clinical Aspects

- **What are eosinophils & what do they do?**
- **Why are eosinophils important to us?**

What are eosinophils & what do they do?

- COMPOSITION
- MATURATION IN BONE MARROW
- RECRUITMENT, ACTIVATION & LOCALIZATION
- EFFECTOR FUNCTIONS

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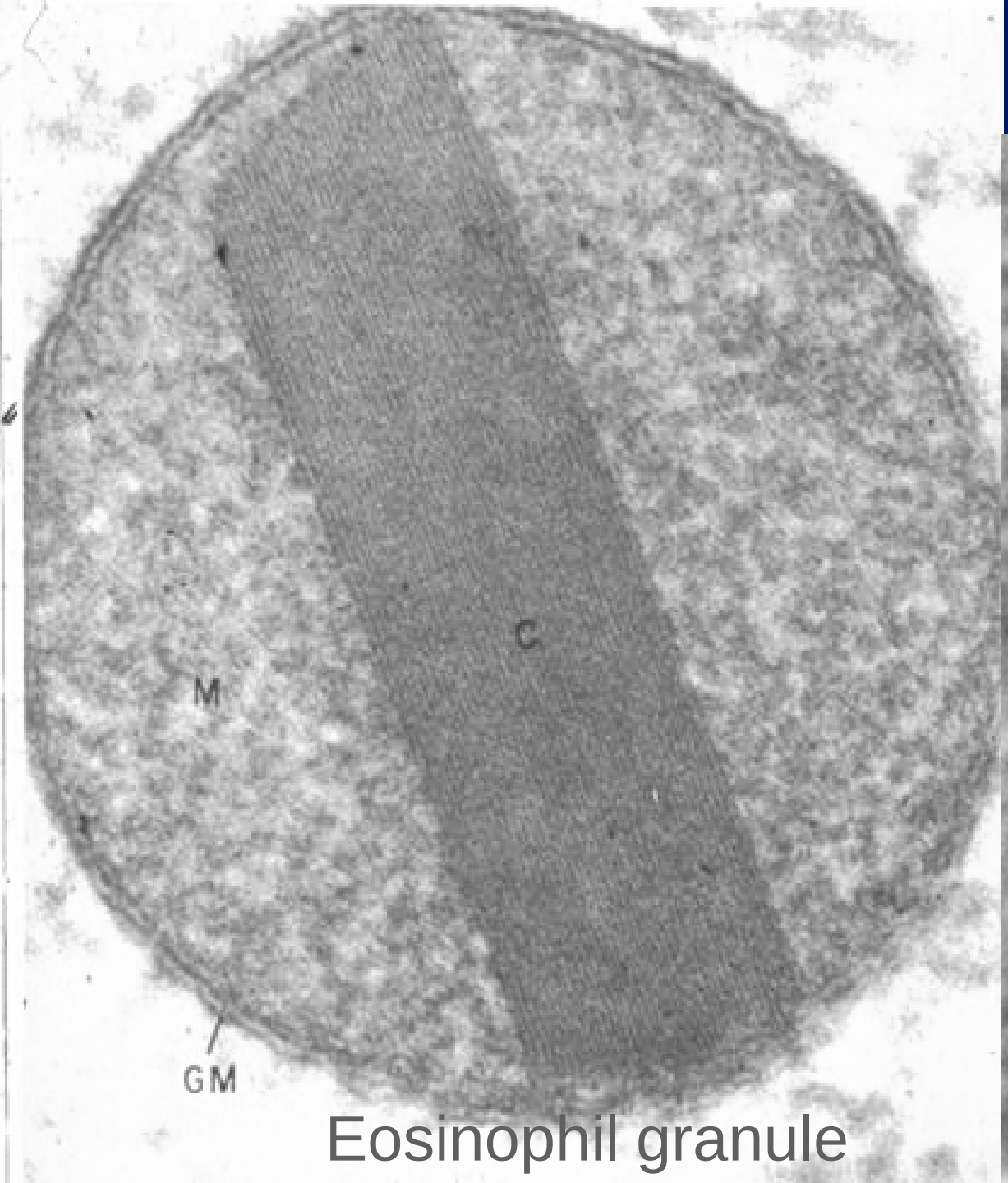
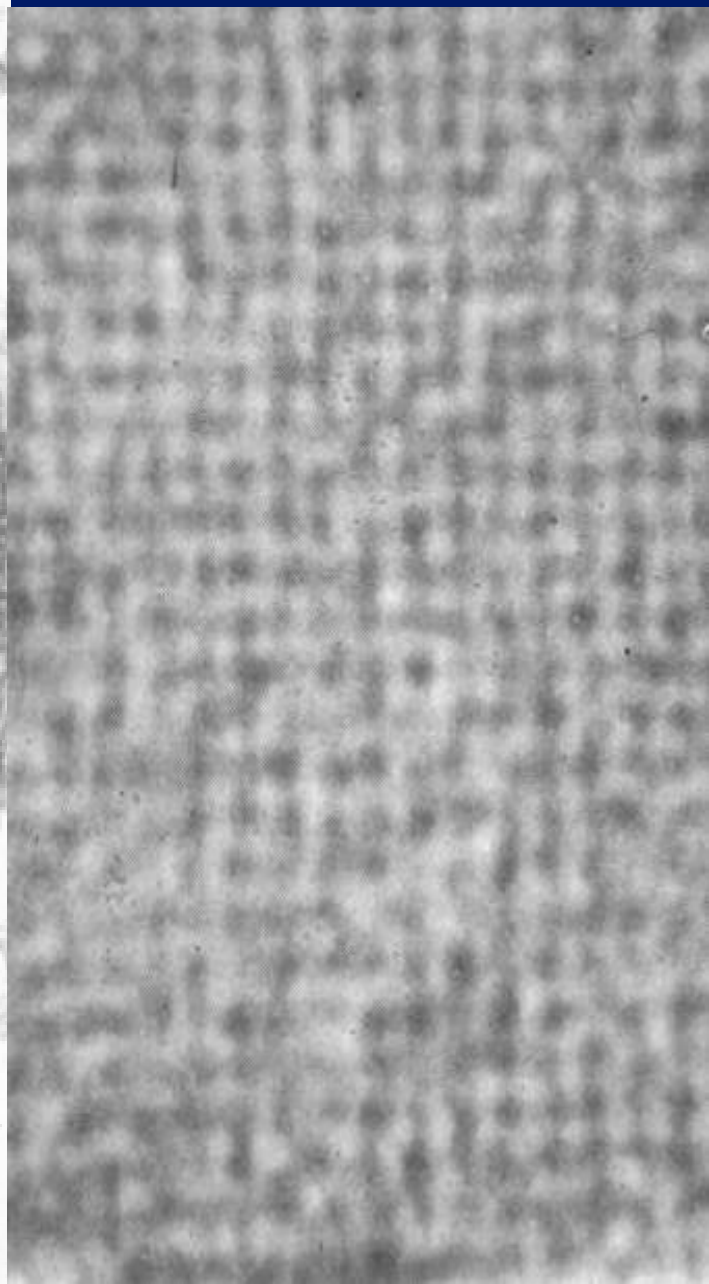


Eosinophil granule

Crystalline core



Secretory Products of Eosinophils

Granule-derived proteins

Major basic protein (MBP1)
Eosinophil cationic protein (ECP)
Eosinophil-derived neurotoxin (EDN)
Eosinophil peroxidase
MBP homolog(MBP2)

Reactive oxygen intermediates

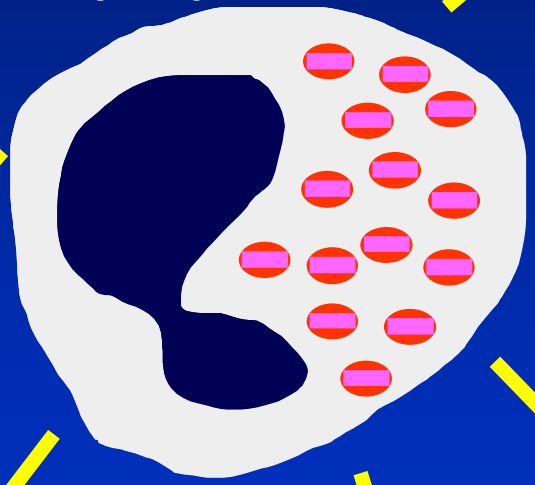
O_2^-
H_2O_2
Hydroxyl radicals
Singlet oxygen

Enzymes

Collagenase
92 kDa Gelatinase (MMP-9)
EPO-dependent brominating oxidants

Lipid mediators

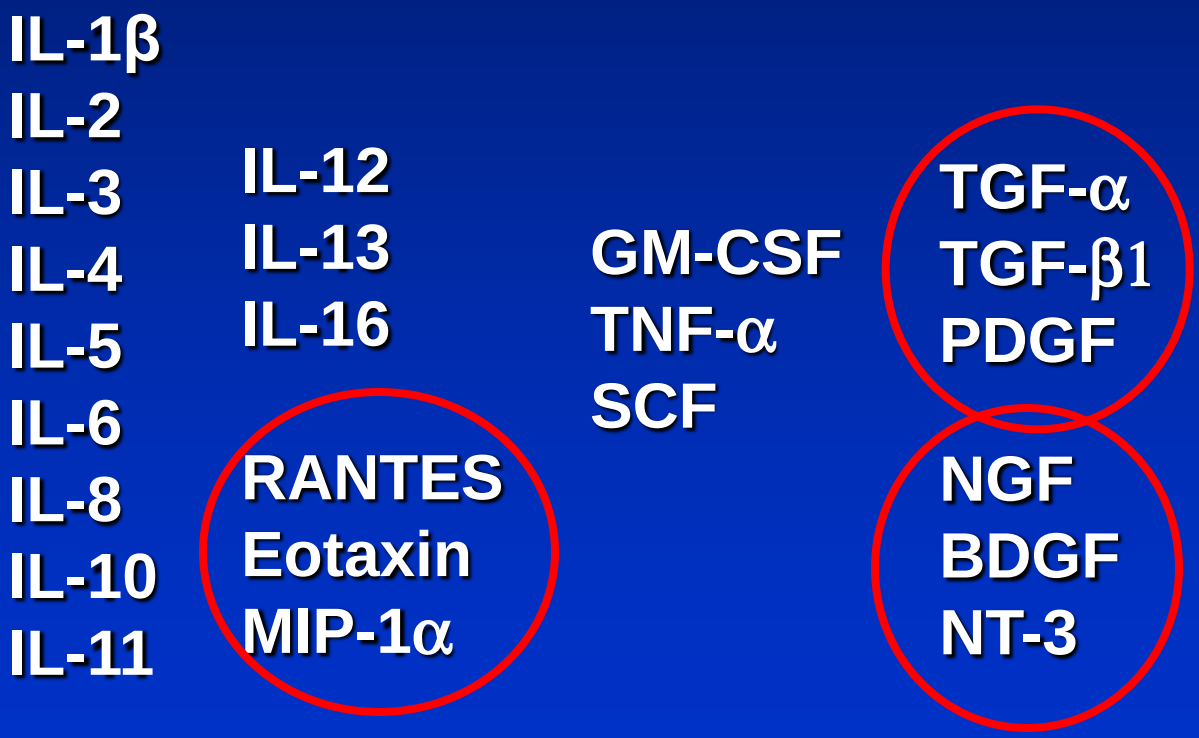
Leukotriene C4/D4
Platelet activating factor
5-HETE
5,15- and 8,15-diHETE
Prostaglandin E_1, E_2
Thromboxane B2



Cytokines

Antigen presentation

Numerous Cytokines And Growth Factors Are Produced by Eosinophils



What are eosinophils & what do they do?

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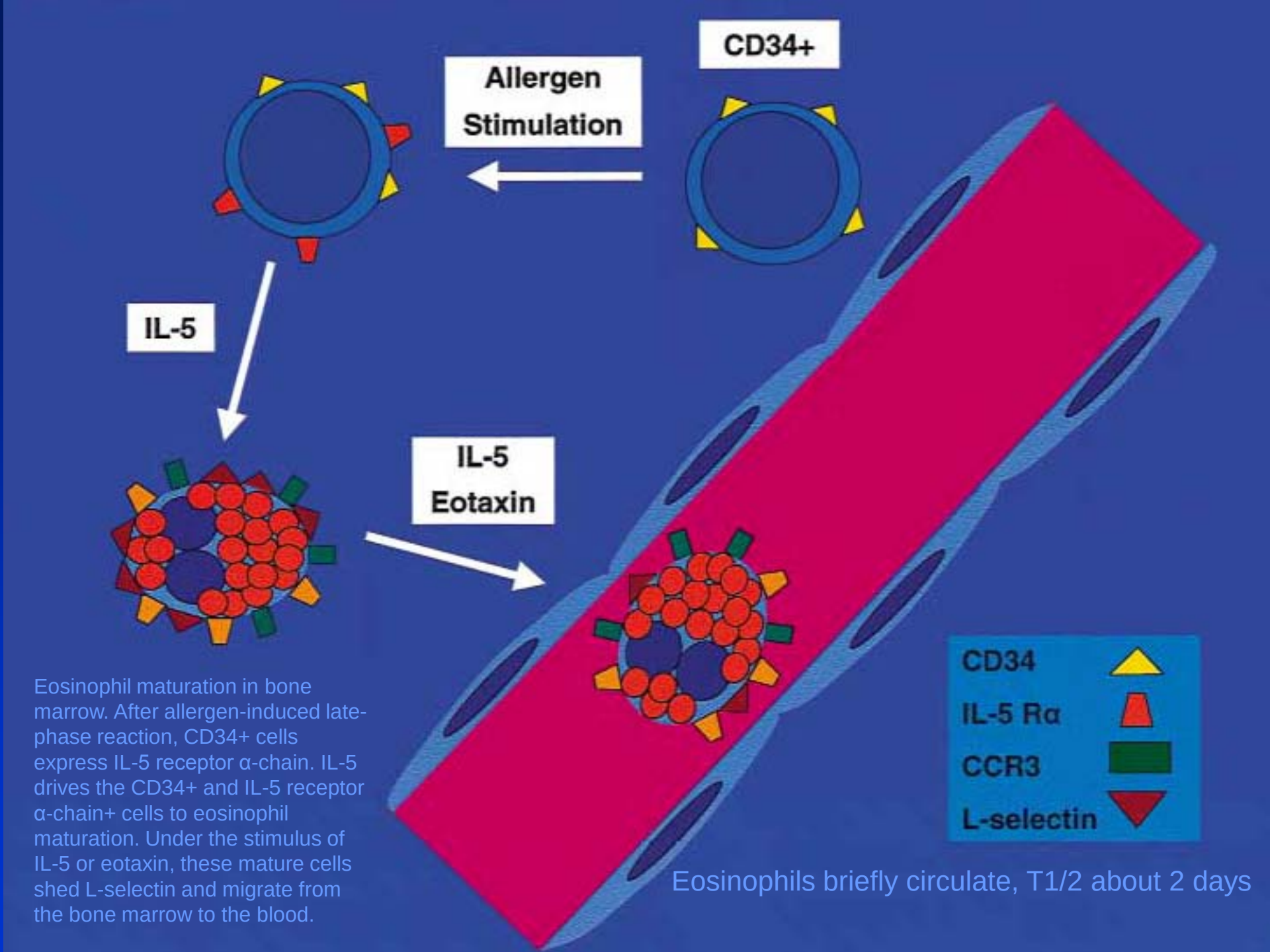

Eosinophil maturation in bone marrow. After allergen-induced late-phase reaction, CD34+ cells express IL-5 receptor α-chain. IL-5 drives the CD34+ and IL-5 receptor α-chain+ cells to eosinophil maturation. Under the stimulus of IL-5 or eotaxin, these mature cells shed L-selectin and migrate from the bone marrow to the blood.

Eosinophils briefly circulate, T1/2 about 2 days

What are eosinophils & what do they do?

- COMPOSITION
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1
2
3
4
5
6
7

Eosinophil Recruitment to and Activation in Bronchial Tissues

At *1*, eosinophils are moving in the peripheral blood.

At *2*, it has tethered by adhesion molecules (e.g., endothelial P-selectin & eosinophil very late activation antigen [VLA]-4 with endothelial vascular cell adhesion molecule [VCAM]-1) and rolls.

At *3*, the eosinophil has firmly adhered to the endothelium (by β1- and β2-integrins & the endothelial ICAM) and has flattened on the surface.

At *4*, the eosinophil is undergoing transmigration between (? through) endothelial cells into the connective tissues.

At *5*, eosinophils (via VLA-4 and VLA-6) interact with connective tissue matrix proteins (eg, fibronectin and laminin), become “primed” and partially activated due to IL-5, RANTES & eotaxin, & lipid mediators such as PAF, and migrate.

At *6*, eosinophils become fully activated by cytokines, lipid mediators, Igs, complement fragments, and β2 integrins and start to degranulate.

At *7*, the eosinophils have degranulated, releasing toxic mediators (eg, granule proteins, lipid mediators, oxygen metabolites, proteases, and cytokines) and disrupting cells in the bronchial epithelium.

Eosinophil Infiltration and Degranulation in Normal Tissues

Very minimal (or no) infiltration or degranulation		Infiltration only	Infiltration and degranulation
Nervous	Muscle	Spleen	Gastrointestinal
Endocrine	Esophagus	Lymph node	Bone marrow
Circulatory	Liver	Thymus	
Respiratory	Pancreas		
Cutaneous	Joint		
Ophthalmic	Gallbladder		
Connective	Placenta		
Genitourinary			

Kato M, Kephart GM, Talley NJ, Wagner JM, Sarr MG et al: Eosinophil infiltration and degranulation in normal human tissue. Anat Record 252:418-425, 1998

What are eosinophils & what do they do?

- COMPOSITION
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EFFECTOR FUNCTIONS

- HEALTH & THE EOSINOPHIL
- VIRAL INFECTIONS
- BACTERIAL KILLING
- HELMINTH KILLING
- FIBROSIS
- IMMUNE MODULATION

Eosinophils & Health

- IL-5 deficient mice are healthy and enjoy a normal life span *
- Eosinophil deficient mice (PHIL) also enjoy a normal healthy and a normal life span #
- No recent reports of humans with eosinophil deficiency

* Foster P. Personal communication. # Lee J. Personal communication

EFFECTOR FUNCTIONS

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- HELMINTH KILLING
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Antigen Sensitization and Viral Infection*

- Infection of sensitized and unsensitized guinea pigs with parainfluenza virus
- Virus multiplied in lungs of unRxed animals
- But viral content of the lung ↓ in sensitized animals
- Reduction in viral content dependent on IL-5 and eosinophils

*Adamko, DJ, et al, J Exp Med 190: 1465, 1999

EFFECTOR FUNCTIONS

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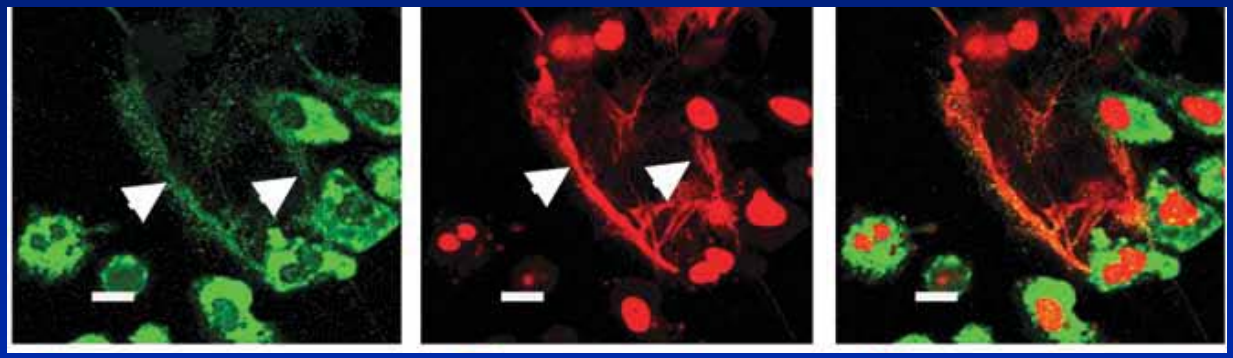
EOSINOPHILS & BACTERIA

- LPS activates primed eosinophil (IL-5) to release mitochondrial DNA
- DNA release dependent on ROS
- DNA release very rapid—less than 1 sec
- In the extracellular space DNA & granule proteins form structures able to bind and kill bacteria
- Murine cecal ligation & puncture model used to show eosinophil protection against sepsis

Yousefi S et al. Catapult-like release of mitochrondrial DNA by eosinophils contributes to antibacterial defence. Nat Med 2008;14:949

EOSINOPHILS & BACTERIA

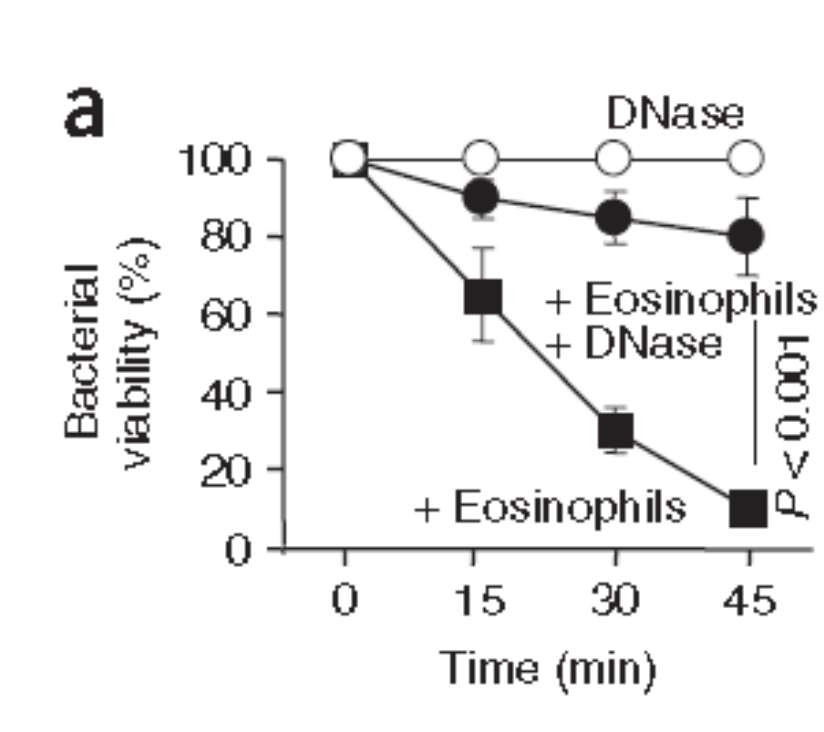
MBP Stain DNA Stain Overlay



Activated eosinophils (IL-5 & C5a) stained for MBP1 & DNA. Overlay shows colocalization of DNA & MBP1. Extracellular deposition of DNA & MBP1 shown by arrowheads. Scale bar= 10 µM.

Yousefi S et al. Catapult-like release of mitochrondrial DNA by eosinophils contributes to antibacterial defence. Nat Med 2008;14:949

EOSINOPHILS & BACTERIA



Killing of E. coli by activated eosinophils (IL-5 & LPS). 100% viability = # colonies formed in absence of eosinophils. Killing was almost completely abolished in the presence of DNase

Yousefi S et al. Catapult-like release of mitochrondrial DNA by eosinophils contributes to antibacterial defence. Nat Med 2008;14:949

EFFECTOR FUNCTIONS

- HEALTH & THE EOSINOPHIL
- VIRAL INFECTIONS
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- **HELMINTH KILLING**
- FIBROSIS
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Eosinophils & Helminths

- Data on importance of the eosinophil in murine helminth infection is conflicting
  - IL-5 deficient mice have diminished resistance
  - PHIL mice have increased resistance
  - Helminths may adapt to presence IL-5/eosinophil
- These findings suggest that while the eosinophil is a specialized cell important in helminth infection, the outcome of the interaction is complex (& parasites may have adapted to the presence of IL-5 & eosinophils)

EFFECTOR FUNCTIONS

- BRONCHIAL HYPERREACTIVITY
- VIRAL INFECTIONS
- BACTERIAL KILLING
- HELMINTH KILLING
- FIBROSIS
- IMMUNE MODULATION

Fibrosis & the Eosinophil

- Association between fibrosis & eosinophilia/ eosinophil degranulation in numerous fibrosing diseases.
- In asthma, expression of TGFβ by eosinophils related to pulmonary function
- In an asthma model eosinophil-deficient mice protected from peribronchiolar collagen deposition & increases in airway smooth muscle.

EFFECTOR FUNCTIONS

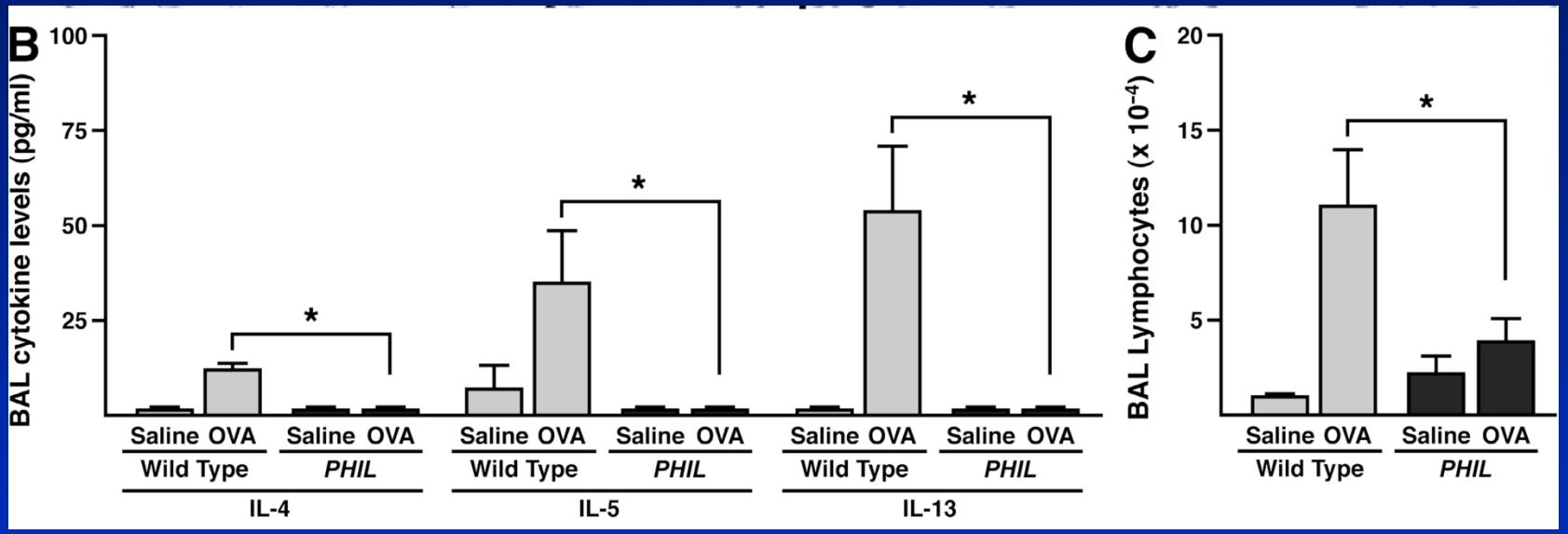
- HEALTH & THE EOSINOPHIL
- VIRAL INFECTIONS
- BACTERIAL KILLING
- HELMINTH KILLING
- FIBROSIS
- **IMMUNE MODULATION**

Eosinophil Immune Modulation

- Eosinophil-less mice (PHIL) show ↓ TH2 airway cytokine levels (and)
- Reduced numbers of airway effector T cells
- Adoptive transfer of antigen-specific T cells & eosinophils corrected airway cytokine levels and lymphocytes in PHIL
- Chemokine production (TARC & MDC), important for T cell recruitment, are eosinophil dependent

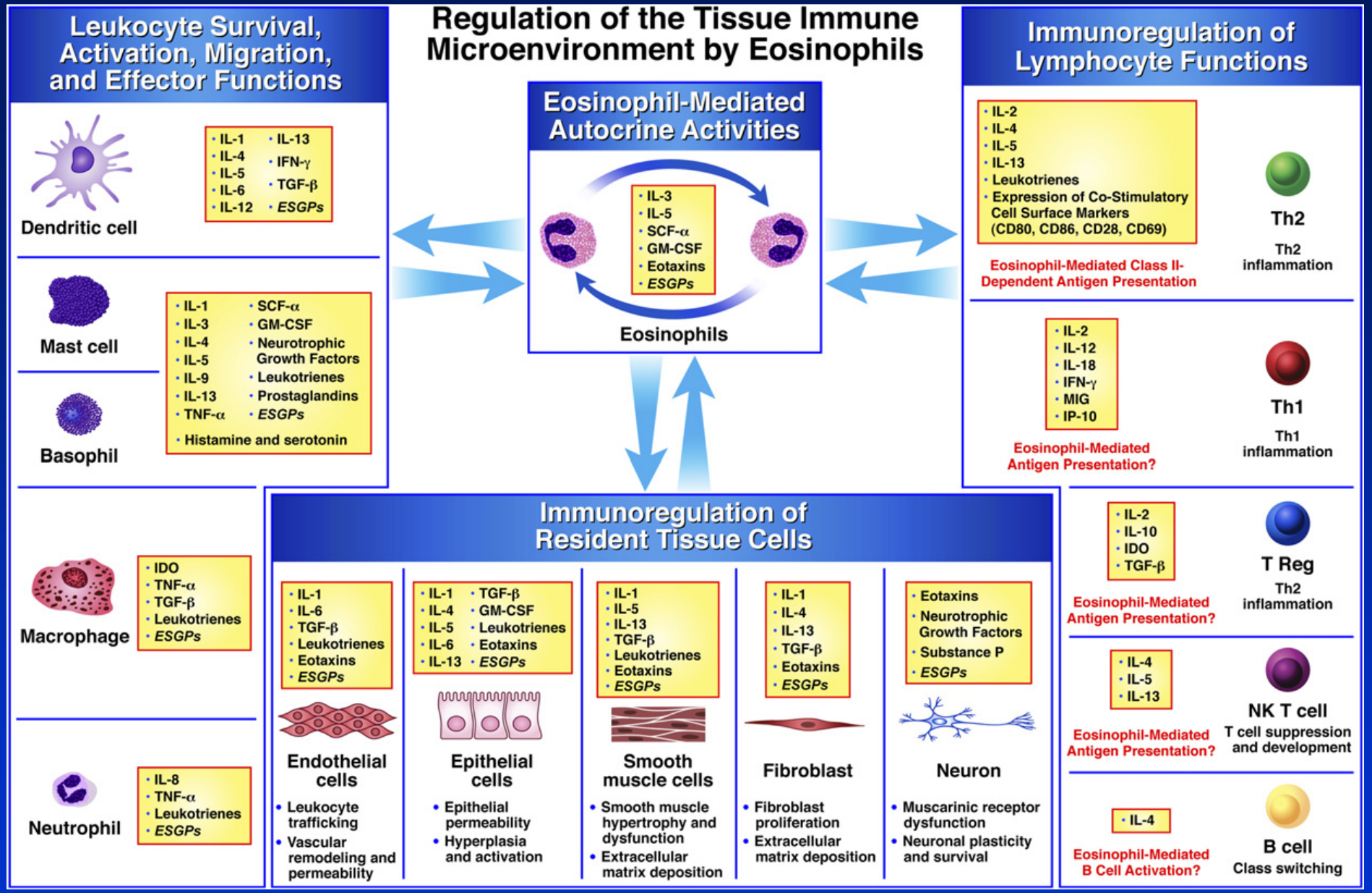
Jacobsen EA et al. J Exp Med 2008;205:699

Eosinophil Immune Modulation



Wild-type & PHIL (eosinophil-less mice) immunized with ovalbumin (OVA). Cytokines measured by ELISA.

Jacobsen EA et al. J Exp Med 2008;205:699



Jacobsen EA et al J Allergy Clin Immunol 2007;119:1313

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Why are eosinophils important to us?

- Granule proteins & activities
- Diseases associated with eosinophilia & eosinophil degranulation
- Asthma & the eosinophil
- Eosinophil-associated diseases & their management

Major Basic Protein: Physicochemical Properties

- Mr ~14,000
- pI 11.4 (by calculation)
- cDNA specifies preproMBP
- Homology & crystal structure, atypical member of C-type lectin superfamily
- Gene, 3.3 Kb, 5 intron, 6 exons
- Site, comprises the granule core

MBP:Biological Properties

- Potent toxin for mammalian cells
- Bactericidal & helminthotoxic
- Toxic to respiratory epithelium causing ciliostasis & desquamation
- Stimulates basophil histamine release
- Potent platelet agonist

MBP:Biological Properties

- Activates neutrophils
- Potent vasodilator
- Causes bronchospasm
- Provokes bronchial hyperreactivity
- Antagonist of inhibitory M2 muscarinic receptors

Why are eosinophils important to us?

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Diseases Associated Eosinophil Degranulation

- Helminths
- Bronchial asthma
- Eosinophilic pneumonia
- Churg-Strauss syndrome
- Allergic bronchopulmonary aspergillosis
- Allergic rhinitis
- Chronic rhinosinusitis & allergic fungal sinusitis
- Nasal polyps
- Episodic angioedema associated with eosinophilia
- Syndromes associated with urticaria & angioedema & the IgE-mediated late phase reaction
- Eosinophilia myalgia syndrome
- Spanish toxic oil syndrome
- Ocular diseases, such as vernal conjunctivitis & giant papillary conjunctivitis
- Renal & hepatic allograft rejection
- Interstitial nephritis

Diseases Associated Eosinophil Degranulation

- Eosinophil endomyocardial disease
- Acute necrotizing myocarditis
- Hodgkin's disease & other lymphomas
- Fibrosis syndromes, such as eosinophilic fasciitis, retroperitoneal fibrosis, sclerosing cholangitis, Riedel's fibrous thyroiditis and orbital pseudotumor
- Eosinophilic cystitis
- Atopic dermatitis
- Onchocercal dermatitis
- Eosinophilic myositis
- Eosinophilic gastroenteritis
- Eosinophilic esophagitis
- NERDS syndrome
- Wegener's granulomatosis
- Paracoccidioidomycosis
- Ulcerative colitis & Crohn's disease
- Myiasis (Hypoderma lineatum)

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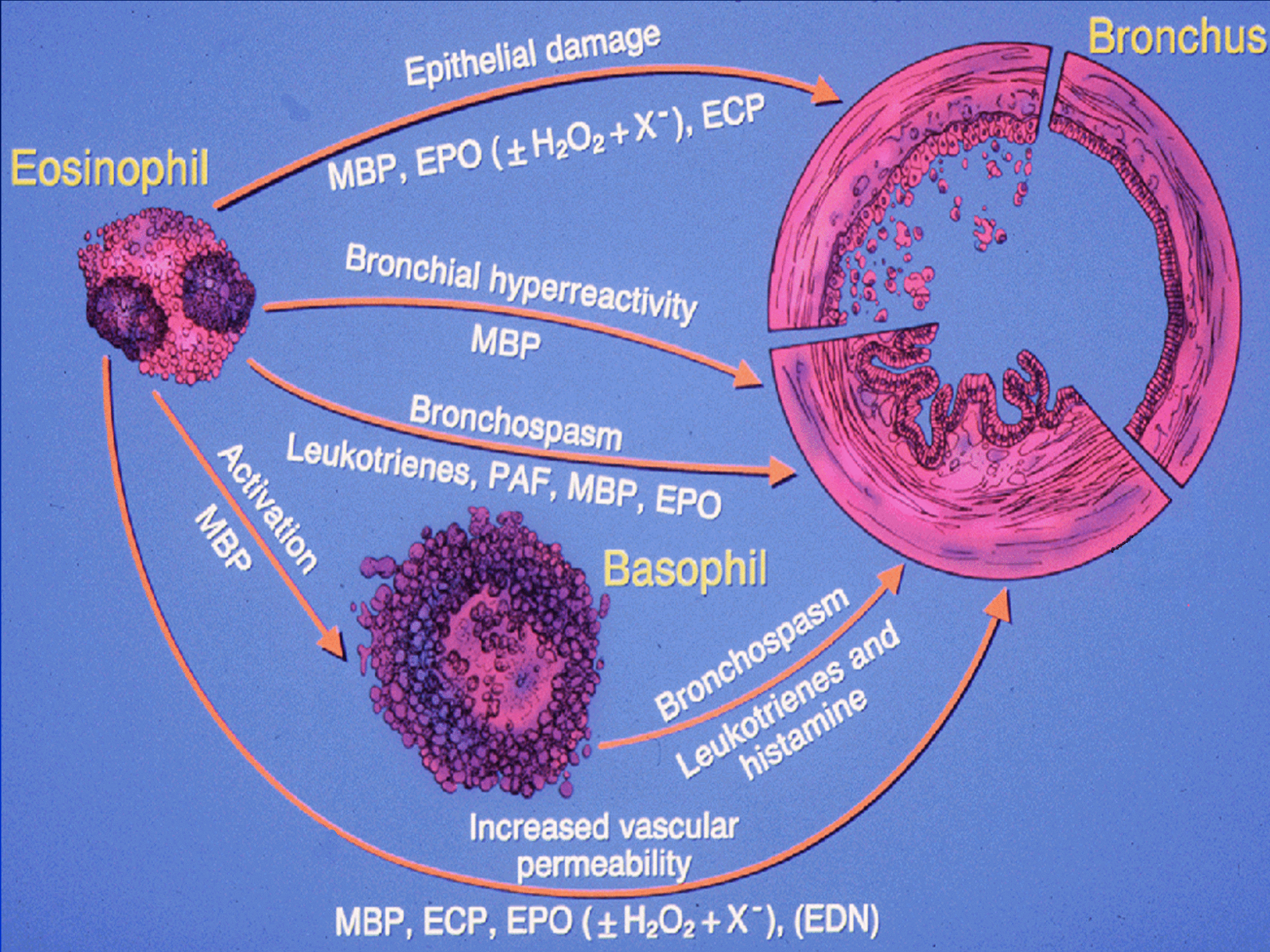

Epithelial damage
MBP, EPO (± H_2O_2 + X^-), ECP
Eosinophil
Bronchus
Bronchial hyperreactivity
MBP
Bronchospasm
Leukotrienes, PAF, MBP, EPO
Activation
MBP
Basophil
Bronchospasm
Leukotrienes and
histamine
Increased vascular
permeability
MBP, ECP, EPO (± H_2O_2 + X^-), (EDN)

Defining a Link with Asthma in Mice Congenitally Deficient in Eosinophils

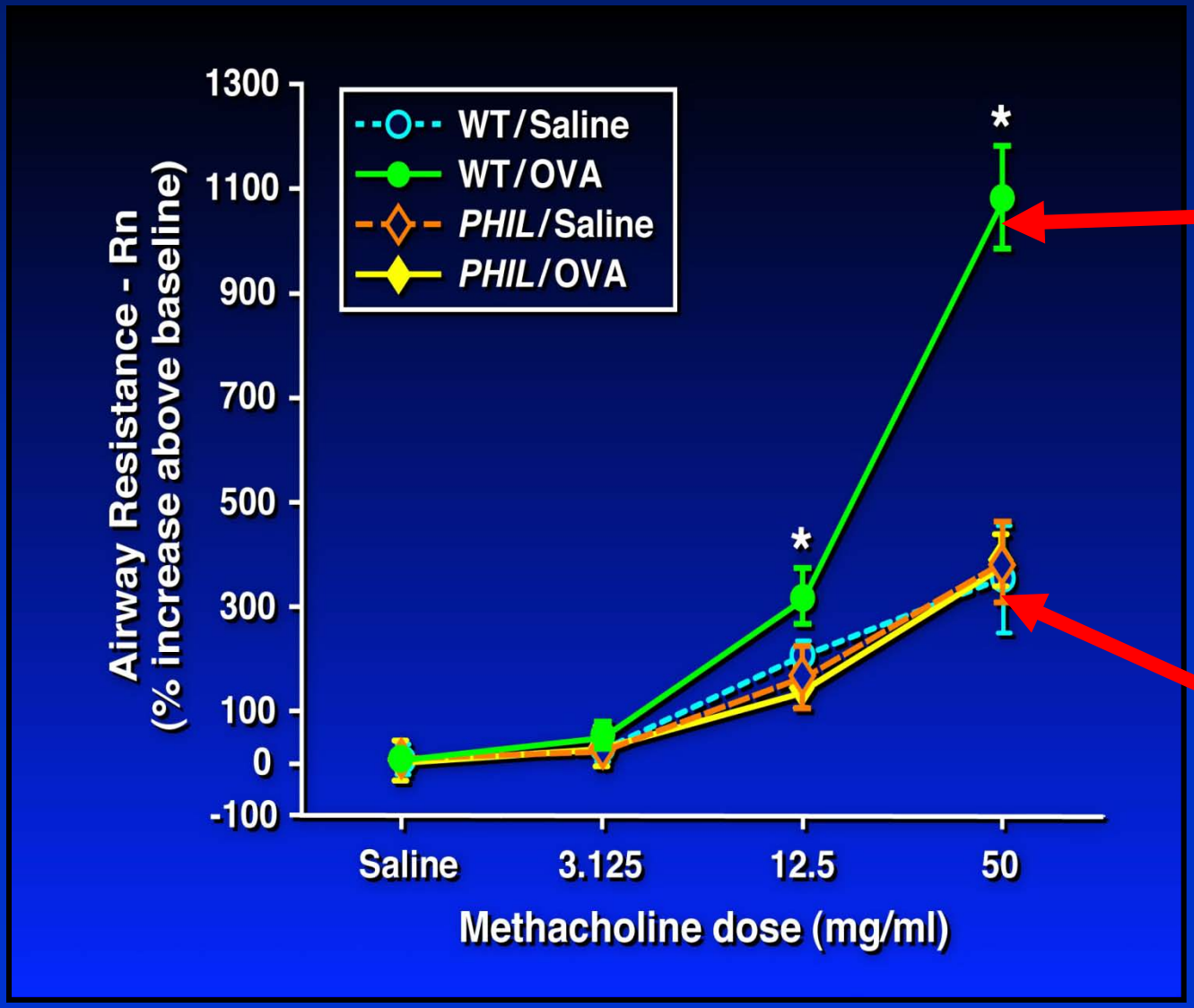
The authors created a transgenic line of mice (PHIL) that are specifically devoid of eosinophils, but otherwise have a full complement of hematopoietically derived cells.

Allergen challenge of these mice demonstrated that eosinophils were required for pulmonary mucus accumulation and the airway hyperresponsiveness associated with asthma.

The development of an eosinophil-less mouse now permits an unambiguous assessment of murine models of many human diseases that have been linked to this granulocyte.

Lee JJ, Dimina D, Macias MP,Ochkur S, McGarry MP et al. Science 2004;305:1773

In the complete absence of eosinophils, ovalbumin sensitization/aerosol challenge-induced airways hyperresponsiveness does not develop



WT asthmatic mouse

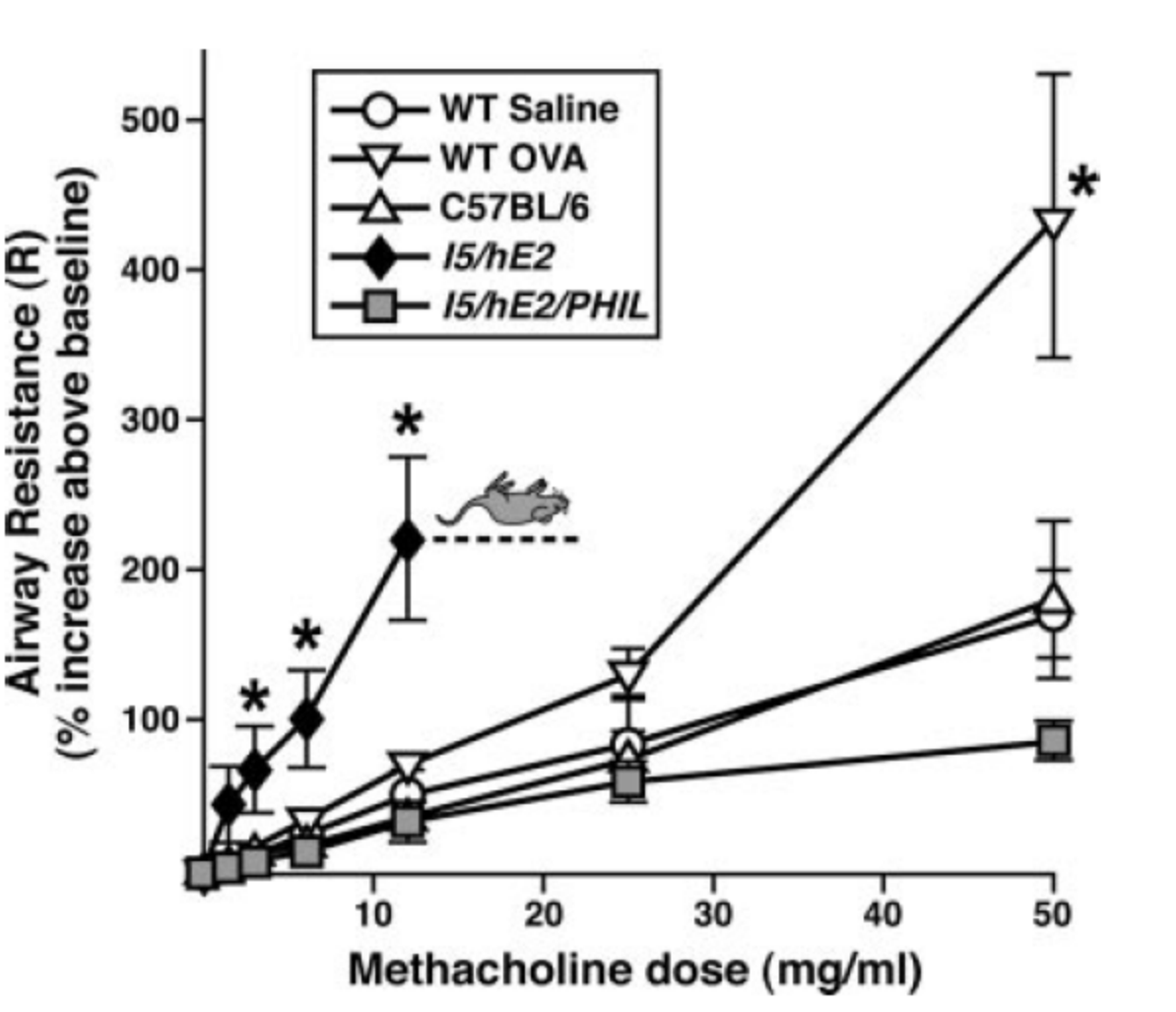
PHIL mouse:"I'm cured!"

Eosinophil-Dependent Model of Severe Asthma

- Double transgenic mouse expressing IL-5 systemically (T cells) & eotaxin-2 (lung epithelial cells)
- Extensive eosinophil infiltration & degranulation
- Development of epithelial desquamation & mucus hypersecretion leading to airway obstruction, subepithelial fibrosis, smooth muscle hyperplasia (antigen independent)

Ochkur SI et al. J Immunol 2007;178:7879

Bronchial Hyperresponsiveness in Transgenic mice



IL-5/Eotaxin (*I5/hE2*) tg C57Bl mice develop extreme BHR often with fatality. BHR totally reversed in eosinophil-less mouse (PHIL)

Ochkur SI et al. J Immunol 2007;178:7879

Why are eosinophils important to us?

- Granule proteins & biological activities
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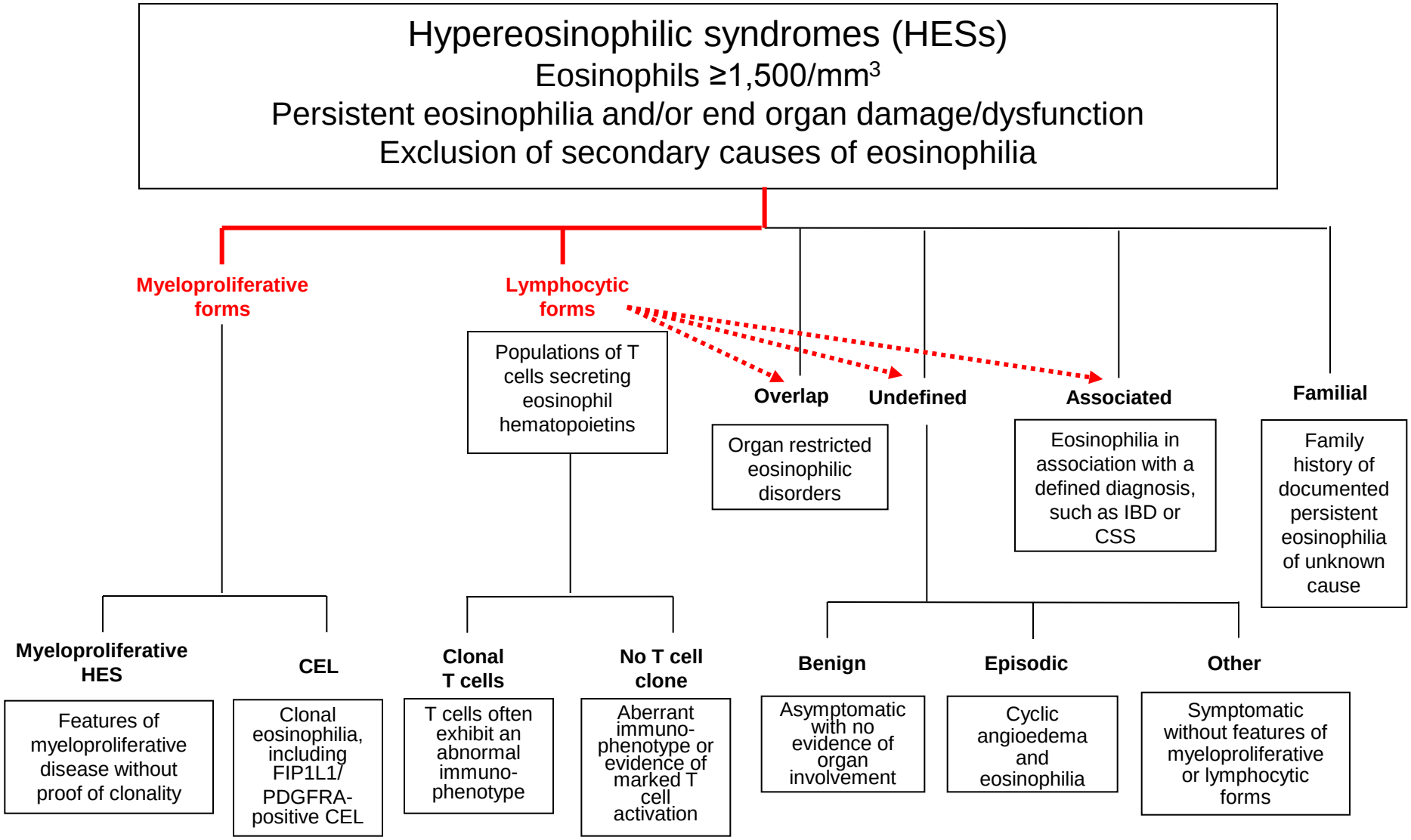
- Asthma & the eosinophil
- **Eosinophil-associated diseases & their management**



CEL, chronic eosinophilic leukemia. IBD, inflammatory bowel disease.
CSS, Churg-Strauss syndrome.

HES Evaluation

- Hx (attention to travel)
- PExam (Spleno-hepatomegaly)
- Repeated eos counts & exam of smear for immaturity
- Stool for O & P
- ESR, CRP
- ANCA
- IgG, IgA, IgM, IgE levels
- Liver enzymes
- SPEP & immunofixation
- Urinalysis
- Echocardiogram
- CAT scan of chest, abd & pelvis
- Tryptase
- Cytoflow (for T cell clones)
- T cell gene rearrangements
- Cytogenetics
- B cell clonality by PCR
- Vitamin B12 level
- Bone marrow aspirate & biopsy (exam for abnormal mast cells) (reticulum stain)
- FIP1L1-PDGFRA (FISH or RT-PCR)
- Strongyloides IgG ab level
- IL-5 serum level
- Biopsy of accessible lesions with immunostaining for eos granule proteins

Therapies for Eosinophil-Associated Diseases

- Glucocorticoids
- Imatinib mesylate
- Peginterferon alfa
- Alumtuzumab (anti-CD52)
- Hydroxyurea
- Humanized monoclonal antibodies to IL-5
- Humanized monoclonal antibody to the IL-5 receptor α-chain (present on eosinophils & basophils)

Eosinophil-associated Disease Rx with Anti-il5

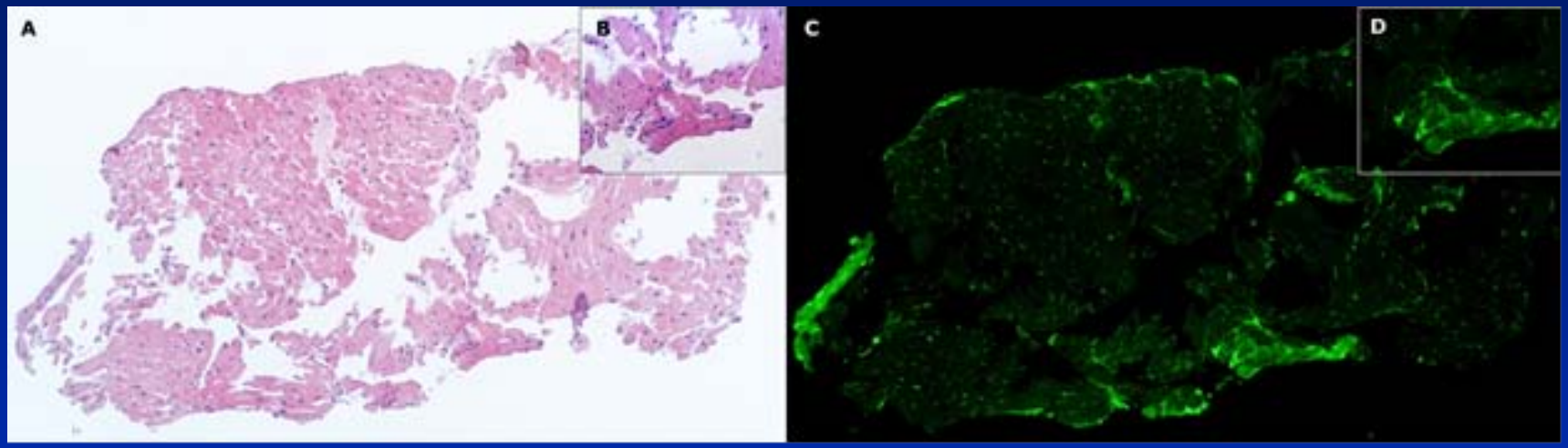
- Use of an Anti–Interleukin-5 Antibody in the Hypereosinophilic Syndrome with Eosinophilic Dermatitis. Plötz s. et al. N Engl J Med 2003; 349:2334, 2003
- Treatment of Patients with the Hypereosinophilic Syndrome with Mepolizumab. Marc E. Rothenberg M.E. N Engl J Med 2008; 358:1215, 2008
- Mepolizumab for Prednisone-Dependent Asthma with Sputum Eosinophilia. Nair P et al. N Engl J Med 2009; 360:985, 2009
- Mepolizumab and Exacerbations of Refractory Eosinophilic Asthma. Haldar P et al. N Engl J Med 2009; 360:973, 2009

28 yo female. Recurrent facial edema, asthma, allergic rhinitis, nasal polyps & eosinophilia. Eosinophilia responsive to 20-40 mg prednisone daily, but unresponsive to imatinib, interferon-alfa, hydroxyurea, methotrexate and diphenhydramine (200 mg/day). Eosinophils 6 X 10^9/L while on 10 mg prednisone daily.

Entered into mepolizumab trial, received placebo but not able to reduce prednisone. Switched to open label administration of mepolizumab and eosinophilia and symptoms controlled. Prednisone discontinued. Presently receives mepolizumab 750 mg IV every 18 weeks with control of eosinophilia and symptoms. Has received mepolizumab since October 2004 without significant difficulty attributable to the medication.

26 y/o ♂. Eosinophilia on health checkup. Eosinophils 3.0 to 16.7 x10^9/L without Rx. Karyotype 46 XY. Cytoflow: no T or B cell abnormality. Three years later fatigue and night sweats. ECHO, & CT chest and abdomen normal. Normal karyotype. Skin Bx of erythematous lesion showed eosinophilia and extensive degranulation. Imatinib Rx 100 mg per day and later per week abolished eosinophilia. 10 years later: still on imatinib at 100 mg/day. Increased erythrocytes.

70 yo male. Eosinophilia & splenomegaly. Episode with transient arm weakness, visual disturbances, disequilibrium and anomia. Brain MRI small embolic stokes. ECHO normal. Rx with prednisone and imatinib ineffective. Cardiac Bx devoid of eosinophils but evidence of degranulation. Rx with pegylated interferon-alfa up to 180 µg per week (with short term administration of hydroxyurea) controlled eosinophilia. Now on 45 µg per 2 weeks with good control. Spenomegaly reduced.



H&E stains of endomyocardial biopsy at 100x (panel A) and 400x (Panel B) magnifications notable for paucity of eosinophils. Staining of same sectionn with antibody to MBP1 (panels C and D) demonstrate striking MBP1 deposition.

35-year-old man. Multisystem illness with striking eosinophilia, painful muscles and pericardial and pleural effusions (CT scan). Rigors, night sweats and fever. Testicular swelling and pain developed. Muscle skin bxs: eosinophil infiltration. 5 months after onset, pleural and pericardial rubs were detected. Chest CT bilateral pleural effusions and moderate pericardial effusion. Hypereosinophilic syndrome was diagnosed and prednisone, 60 mg daily, was started. New skin lesion developed and patient rubbed the pruritic lesion.

This crawled out of the lesion. Identified as Hypoderma lineatum, second instar stage

Prednisone was rapidly tapered and discontinued. 2 weeks later another larva exited from a skin lesion on the patient’s back

Myiasis due to *Hypoderma lineatum*

- Larvae from the cattle grub, Hypoderma lineatum, rarely infect humans
- Impressive blood and tissue eosinophilia can develop
- Multisystem disease resembling the hypereosinophilic syndrome or eosinophilia myalgia syndrome
- Symptoms resolve with egress of larvae

Myiasis due to *Hypoderma lineatum*

- Myiasis—the infestation of human and vertebrate animals with the larvae of dipterous flies, which feed on the host's dead or living tissue, liquid body substances or ingested food
- Symptomatic cutaneous, ophthalmologic, nasal, aural, vaginal, gastrointestinal and pleural involvement (cutaneous most common)

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Eos, Greek goddess of dawn