Neutrophils: lessons learned from patients

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Objectives

- Clinical manifestations of neutrophil defects
- Molecular basis
- Focus will be on primary rather than iatrogenic neutrophil defects
General principles of immunity

• Our immune system evolved to defend against pathogens
  – Endogenous flora
  – Inhaled
  – Traumatic inoculation
  – Communicable

• Our immune system is not fine-tuned for major insults (e.g., trauma, sepsis) that aren’t survivable in nature

• Cancer: innate immune responses can promote tumor control or progression
Basic requirements of phagocytes

• Adequate numbers
• Migrate to where they are needed
• Sense and respond to microbes
• Sense and respond to inflammatory mediators (e.g., cytokines, chemokines)
• Kill pathogens
• Mechanisms to “turn off” inflammation once pathogen threat has been eliminated
When to think of a primary phagocytic disorder?

• Usually (not always) first manifest during childhood
• Unusually severe or recurrent infection by common pathogens
• Opportunistic pathogens
Neutrophil disorders

• Quantitative

• Qualitative phagocytic disorders
  – NADPH oxidase (CGD)
  – Adhesion (Leukocyte adhesion deficiency)
  – Granular deficiencies (Chediak-Higashi, specific granular deficiency)
  – Signaling (Job’s)
Neutropenia: causes

• Primary disorders of granulopoiesis (uncommon)
• Hematological cancers (e.g., leukemia) and disorders leading to marrow failure (e.g., myelodysplastic syndrome, aplastic anemia)
• Drug-induced (e.g., chemotherapy)
• Autoimmune
Patients with invasive pulmonary aspergillosis %

DURATION OF GRANULOCYTOPENIA (DAYS)

PATIENTS AT RISK
Neutrophil disorders

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  – Unspecified deficiency (e.g., Job’s)
How do neutrophils kill pathogens?
How do neutrophils kill pathogens?

- Neutrophils kill microorganisms through oxygen-independent and oxygen-dependent mechanisms
- Oxygen-independent mechanisms include the release of peptides and proteins from the granules, such as bactericidal permeability-increasing proteins (BPI), defensins and cathelicidins
Chronic granulomatous disease (CGD)

- Inherited deficiency of NADPH oxidase
- X-linked and AR forms
- Incidence ~ 1 in 250,000
- Major pathogens
  - Staph aureus (deep soft tissue, pneumonia, liver)
  - Nocardia (lung, brain, bone/soft tissue)
  - Burkholderia cepacia (pneumonia)
  - Serratia (bone)
  - Salmonella (sepsis)
  - Aspergillus and other moulds (lung, disseminated)
NADPH oxidase

Activation of primary granular proteases

Cytoplasm

NADPH → NADP⁺ + H⁺

H₂O₂ → O₂⁻ → HOCI

SOD

O₂

MPO

p22phox

gp91phox

p67phox

p47phox

p40phox

p7phox

RhoGDI

FAD

HEME

GTP

GDP
Infectious complications in CGD patients
Neutrophil extracellular proteases (NETs)

- Extracellular structures that are composed of chromatin and proteins from the neutrophilic granules
- NETs are complex structures composed of ‘threads’, approximately 15 nm in diameter, which are likely to represent a chain of nucleosomes from unfolded chromatin
- Antimicrobial properties of NETs are likely from histones, and neutrophil granular antimicrobial peptides and proteases
- NETs are generated following stimulation with several neutrophil activators, e.g. LPS, bacteria, fungi, IL-8, activated platelets
Neutrophil extracellular traps (NETs)

Brinkmann et al. Science, 2004
NETs bind to bacteria and fungi in extracellular space
Gene therapy restores NET formation in neutrophils from CGD patient

-- PMA stimulation (3 hours)
-- staining for neutrophil elastase (green)

NETosis during pulmonary aspergillosis requires NADPH oxidase

Rohm et al Infect Immune, 2014
Inflammatory complications of CGD
Inflammatory response in wild-type and X-CGD mice lungs 21 d after challenge with sterile *A. fumigatus* hyphae

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**Wildtype**

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**X-CGD**

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Increased lung inflammation in CGD mice after i.t. zymosan

Innate Immunity against Aspergillus

Dectin-1

- C-type lectin
- binds to yeast and signals through the kinase Syk and the adaptor CARD9
- Dectin-1-activated DCs prime the differentiation of TH-17 cells \textit{in vitro}
- Deficiency in Dectin-1/CARD9 signaling linked to familial mucocutaneous candidiasis

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Neutrophil trafficking

http://www.youtube.com/watch?v=WEGGMaRX8f0&feature=player_detailpage
Skin Infection in LAD I

- Minimal to no erythema
- Failure to form pus at the site of infection
- Healing with dysplastic scars
- Often requiring prolonged antibiotic course and surgical debridement
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NUCLEUS

PRIMARY (AZUROPHILIC) GRANULES

GIANT (FUSED) LYSOSOME OF CHEDIAK-HIGASHI SYNDROME

SECONDARY (SPECIFIC) GRANULES

(Absent in Neutrophil Specific Granule Deficiency)
Chediak-Higashi syndrome

- severe autosomal recessive condition of *LYST* gene (lysosomal trafficking regulator)
- partial oculocutaneous albinism with photophobia
- increased susceptibility to infections (mostly *Staphylococcus* and *streptococci*)
- Neurologic manifestations
- Most patients who don’t undergo stem cell transplantation die of a lymphoproliferative syndrome
  - widespread visceral infiltration of lymphohistiocytic cells gives rise to fever, jaundice, hepatosplenomegaly, lymphadenopathy, pancytopenia, bleeding
CHS

-- Abnormally large leukocyte granules result from fusion of lysozymes.
-- may affect granulocytes, leukocytes, and monocytes
-- Chemotaxis and phagocytosis are defective
-- Platelets lack dense granules and platelet function is abnormal.
CHS: occulocutaneous albinism
CHS gene

- Beige mouse gene (*LYST* --> Lysosomal Transport Protein)
- Chromosome 1q42-1q43
- 3801 AA protein --> intracellular trafficking
- Multiple mutations --> truncation identified in CHS patients (Certain et al. Blood, 2000)
Specific Granule Deficiency

- Recurrent infections (no particular class)
- Defective neutrophil chemotaxis and killing
- Neutrophils lack visualized granules on Wright staining, "empty specific granules"
- Bilobed nuclei, clefts, pockets
- Eosinophils lack granules
- Bleeding disorder: ↓ von Willebrand factor multimers in platelet granules
Specific Granule Deficiency

• Reduced or absent subset of neutrophil specific granule proteins
  – lactoferrin
  – vit B12 binding protein
  – procollagenase
  – cytochrome B
  – c3Bi receptor
• Defensins
• Eosinophils also lack granular specific proteins
• Reduced or absent mRNA of these constituents in BM precursors
C/EBPε

C/EBPs (CCAAT/enhancer binding proteins)
- leucine zipper family of transcription factors
- repressors/activators $\Rightarrow$ cellular differentiation

C/EBPε: expressed primarily in myeloid cells
Defective in patients with specific granule deficiency

C/EBPε KO mice recapitualte the human phenotype

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Job’s syndrome (HIESRI)

- Severe eczema starting at infancy
- Deep soft tissue infections (cold abscesses), usually caused by Staph aureus
- Pneumonias (pneumatoceles)
- ↑IgE
- Differentiate from atopic dermatitis by frequency and severity of infections
- Non-immunologic manifestations
  - Delayed shedding of the primary teeth owing to lack of root resorption, recurrent fractures, hyperextensible joints and scoliosis, characteristic facial appearance
Job’s
(Hyper-IgE syndrome with recurrent infections)
Failure of Dental Exfoliation in Patients with the Hyper-IgE Syndrome.
Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome

PBLs from Job’s patients
-- Normal STAT3 levels at baseline and after IFN-α stimulation
-- Decreased DNA binding of STAT3
-- Defective responses to IL-6 and IL-10
-- Decreased Th-17 development

Conclusions

• Primary neutrophil defects are for the most part rare
• Many have extra-immunologic manifestations
• “Experiments of nature” teach us about neutrophil development and host defense pathways
• Opportunities for novel therapeutics (e.g., cytokines, BMT, gene therapy)
General principles of immunity

• Our immune system evolved to defend against pathogens
  – Endogenous flora
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• Cancer: innate immune responses can promote tumor control or progression
Cancer: a wound that doesn’t heal
Microbial products (PAMPs) and endogenous products of necrosis (DAMPs) can activate similar innate immune receptors.

Brennan et al. Journal of Transplantation, 2010
Tumor microenvironment in EOC stimulates NETosis

NETosis in human EOC

Intact neutrophil

Cell-free ascites induces in normal donor neutrophils