White Blood Cell Disorders

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O2 independent killing
acquisition of respiratory burst
phagocytosis
chemotaxis
acquisition of respiratory burst
O2 independent killing

granule mRNA

1\textsuperscript{o} granule mRNA

2\textsuperscript{o} granule mRNA

*
Cytokines Govern Myelopoiesis

- **GROWTH FACTORS/RECEPTORS**
  - Induce transcriptional program governing neutrophil maturation
  - G-CSF/G-CSFr
    - knock-out: relative neutropenia (20% PMN)
  - GM-CSF
    - knock-out: no defect in neutrophil maturation

- **G-CSF FUNCTION IN MYELOPOIESIS**
  - Proliferation of myeloid progenitors
  - Induction of myeloid maturation
  - Protection from apoptosis
  - Enhancement of neutrophil function
Life Span of the Neutrophil

- Maturation in the bone marrow: 7-10 days
- Circulation in the peripheral blood: 3-6 hours
- Duration in the tissues: 2-3 days
### Peripheral WBC Count

<table>
<thead>
<tr>
<th>Pool</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid Precursors</td>
<td>20%</td>
</tr>
<tr>
<td>Storage Pool</td>
<td>75%</td>
</tr>
<tr>
<td>Marginating Pool</td>
<td>3%</td>
</tr>
<tr>
<td>Circulating Pool</td>
<td>2%</td>
</tr>
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Peripheral WBC Count

- Myeloid Precursors: 20%
- Storage Pool: 75%
- Marginating Pool: 3%
- Circulating Pool: 2%

- The peripheral neutrophil count reflects <5% of the total WBC pool during a period of 2% of the total WBC lifespan.

- Elevation of WBC counts:
  - Acute, rapid: changes in distribution (demargination)
  - Long term, chronic elevation: changes in production and release from storage pool

- Decreased WBC counts:
  - Defect in WBC production, increased destruction, or increased margination (sequestration)
A 43-year-old woman with elevated WBC

Previously healthy woman seen for routine office visit is noted to have a WBC 12K, with normal differential.
Repeated three weeks later- no change.
Hct 42; Plts 230K
Leukocytosis: Differential Diagnosis

SECONDARY TO OTHER ILLNESSES

- Infection
  - Acute: Demargination/release storage pool
  - Chronic: Granulomatous dx (leukoerythroblastic)
- Stress
- Drug-induced (steroids, β-agonists, lithium)
- Chronic inflammation (including smoking)
- Post-splenectomy
- Non-hematologic malignancy
- Marrow stimulation (ITP, hemolysis, CMT)
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- PRIMARY HEMATOLOGIC DISEASE
  - CML
  - Other MPD
Neutrophilia is usually reactive, indicative of a normal functioning bone marrow. Consequently, bone marrow evaluation is often unnecessary
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- Repeat WBC to R/O factitious or artifactual elevation
- Evaluation for acute/chronic infection or inflammation
- LAP score-of limited value since bcr-abl testing
- FISH for bcr-abl
- Bone marrow exam: r/o granulomatous dx, fungus
CASE PRESENTATION

A 1-month-old boy with elevated WBC

- 1 month old infant with delayed umbilical cord separation
- High grade fever, MRSA infection, and WBC of 90,000
- Poorly healing skin lesions, otitis, failure to thrive
- Poor response to antibiotics
- What to do??

Adapted from Pediatr Transplantation 11:453-5, 2007
Leukocytosis: Differential Diagnosis

- PRIMARY HEMATOLOGIC DISEASE
  - Congenital
    - Hereditary neutrophilia
    - Down’s sx
    - Leukocyte Adhesion Deficiency
Adhesion Molecules and LAD

Pathogenesis:

- Defective integrin receptor common β chain (LAD I)
- Loss of expression of LFA-1, Mac-1 (C3biR), and gp150;95.
- Results in inability to ingest/kill microbes opsonized by C3bi
- Can also arise by an abnormality of selectin glycosylation that impairs leukocyte adhesion (LAD II)
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Clinical manifestations:

- elevated WBC
- recurrent infections, mainly cutaneous abscesses, gingivitis
- Many die before age 2
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Treatment:
- Stem cell transplant: treatment of choice (performed on the patient described here)
- G-CSF has been tried experimentally
<table>
<thead>
<tr>
<th>Neutrophil Count</th>
<th>Clinical Implications</th>
</tr>
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<tbody>
<tr>
<td>&gt;1500</td>
<td>Normal</td>
</tr>
<tr>
<td>1000-1500</td>
<td>May be normal; no significant increased infection</td>
</tr>
<tr>
<td>500-1000</td>
<td>Some increased risk of infection; fever mx as outpt</td>
</tr>
<tr>
<td>&lt;500</td>
<td>Significant risk of infection; fevers managed w/ iv abx as inpatient; often few signs of infection.</td>
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</table>
<500 Neutrophils: Significant risk of infection in patient with acute neutropenia, chemotherapy induced neutropenia, etc.

- Most patients with chronic neutropenia don’t get into trouble until the count is < 200.

*In chronic neutropenia, patients frequently have little or no manifestations of neutropenia with counts in the 50-100 range.*
A 2-month old girl with agranulocytosis

- 2 mo old girl with fever, purulent otitis, and boils
- FH: 1 of 9 children; 4 had died at a young age
- Cultures + for *S. aureus*. Treated with streptomycin
- Peripheral smear: no granulocytes
- Marrow: maturation arrest at the promyelocyte stage
- Subsequent course: died at age of 6 months despite antibiotics with widespread infection, boils, thrush

Adapted from Kostmann, Acta Paediatr Scand 1956
Neutropenia: Differential Diagnosis

CONGENITAL NEUTROPENIAS

Benign neutropenia
- Constitutional neutropenia
- Benign neutropenia (familial, idiopathic)

Congenital
- Severe congenital neutropenia, including Kostmann’s syndrome

Cyclic neutropenia

Other rare disorders
- Chediak-Higashi
- Schwachmann-Diamond
Severe Congenital Neutropenia

- congenital agranulocytosis
- rare
- autosomal dominant, recessive, and sporadic cases reported.
- severe infections; survival dramatically changed by treatment with G-CSF
- high incidence (20-30% over 10 years) of evolution to AML.
Autosomal dominant form of SCN:

- linked to mutations in the neutrophil elastase (ELANE) gene
- Variable impact of different mutations on enzyme function
- Mutant ELANE accumulates in the cytoplasm, and activates the “unfolded protein response,” a cellular stress response that results in apoptosis.
- AML associated with a truncation mutation of the G-CSF receptor of uncertain pathogenetic significance
Severe Congenital Neutropenia

Autosomal recessive SCN:

- Kostmann’s Syndrome: original syndrome described 50 years ago
- Linked to mutations in HAX1, a mitochondrial protein associated with signal transduction
- Disruption of HAX1 in myeloid cells destabilizes the mitochondrial membrane and leads to apoptosis
Cyclic Neutropenia

- dominantly inherited
- cycle of neutropenia q 15-35 days
- marrow during neutropenia: myelocyte arrest
- Usually benign; patients with severe infections may respond to G-CSF
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- dominantly inherited
- cycle of neutropenia q 15-35 days
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- Usually benign; patients with severe infections may respond to G-CSF
- Like AD SCN, cyclic neutropenia has been linked to mutations in ELANE
- ELANE mutations found in essentially 100% of cyclic neutropenia
- NOT associated with an increased risk of AML
CASE PRESENTATION

38 yo woman with SLE and neutropenia

HPI:
- Age 14: pericarditis, Raynaud’s with prolonged period of bedrest. ?JRA; ?SLE
- Age 26: fatigue, adenopathy, oral ulcers, arthritis. Leukopenia, thrombocytopenia, +ANA, +ACA
- Age 30: miscarriage. Documented ACLA

MEDS:
- Plaquinil, ASA 81mg, Prednisone 5; recent taper from 50mg

EXAM: Malar rash; no active joint disease

LABS: WBC 1.8
Primary AIN:
- Seen primarily in children
- Caused by antibodies against neutrophil ags
- Average age of onset: 6-12 months
- Moderate to severe neutropenia
- Spontaneous remission over 2 yrs: 95%
- Treatment: Prophylactic antibiotics; G-CSF only with severe/recurrent infections
Autoimmune Neutropenia

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**Secondary AIN**
- Seen primarily in adults
- Associated with AID
- Associated with LGL
Occurs in approximately 50% of SLE patients
Marker of disease activity
Little impact on the course of the disease
Infectious complications correlate with immunosuppressive therapy rather than height of neutrophil count
Neutrophil-specific antibodies
  High incidence of neutrophil-associated IgG in SLE
  Poor correlation with neutropenia
# Antineutrophil Antibody Testing

<table>
<thead>
<tr>
<th>ANTIGEN</th>
<th>PREVIOUS NOMENCLATURE</th>
<th>GLYCOPROTEIN</th>
<th>ALLELE FREQUENCY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNA-1a</td>
<td>NA1</td>
<td>FcγIIIb (CD16)</td>
<td>58</td>
</tr>
<tr>
<td>HNA-1b</td>
<td>NA2</td>
<td>FcγIIIb (CD16)</td>
<td>88</td>
</tr>
<tr>
<td>HNA-1c</td>
<td>SH, NA3</td>
<td>FcγIIIb (CD16)</td>
<td>5-38</td>
</tr>
<tr>
<td>HNA-2a</td>
<td>NB1</td>
<td>CD177(gp50-64)</td>
<td>94</td>
</tr>
<tr>
<td>HNA-3a</td>
<td>5b</td>
<td>Gp70-95</td>
<td>97</td>
</tr>
<tr>
<td>HNA-4a</td>
<td>MART</td>
<td>CD11a</td>
<td>99</td>
</tr>
<tr>
<td>HNA-5a</td>
<td>OND</td>
<td>CD11b</td>
<td>96</td>
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Pitfalls of Antineutrophil Antibody Testing

- False positive results
  - abundant Fc receptors on neutrophils
    - high circulating antibody
    - circulating immune complexes
  - spontaneous fluorescence of neutrophils
  - spontaneous aggregation of neutrophils
  - fragility, with spontaneous lysis
Pitfalls of Antineutrophil Antibody Testing

- False positive results
  - abundant Fc receptors on neutrophils
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  - spontaneous fluorescence of neutrophils
  - spontaneous aggregation of neutrophils
  - fragility, with spontaneous lysis
- Effect on outcome remains undefined
  - no “gold standard”
  - non-neutropenic patients often have detectable antibody
  - poor correlation between level of antibody and degree of neutropenia
WHEN DO I CHECK ANTI-WBC ABS ON ADULT PATIENTS?
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NEVER
58 yo man admitted with fever and cellulitis

PMH:
- hypercholesterolemia, NIDDM, arthritis
- Medications: naproxen, glucosamine, simvastatin

PE:
- Multiple joint deformities, splenomegaly, no adenopathy

CBC:
- Hct 40, Plt 200K; WBC 5900 w/90% lymphs, 1% polys
Autoimmune neutropenia in RA

Felty’s syndrome

- Typically in patients with longstanding RA
- Associated with end-organ RA manifestations (pulmonary fibrosis, vasculitis, rheumatoid nodules, Sjogren’s syndrome)
- Splenomegaly
- Considerable morbidity from bacterial infection
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LGL-associated neutropenia
- Shares many features with Felty’s syndrome
- Monoclonal neoplastic disorder, while Felty’s traditionally is polyclonal
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*Both have a very high (90%) incidence of HLADR4, suggesting they are a spectrum of the same disease*
65 yo man with sore throat and fever

PMH: chronic CHF and has been taking several cardiac drugs for 2 months

CBC: Hb 12; Plts 190K; WBC 0.7 with ANC 50
### Drugs most commonly causing Agranulocytosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-thyroid medications</td>
<td>Carbamizole, Methimazole, Thiouracil</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Cephalosporins, Penicillins, Sulfonamides, Chloramphenicol</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>Carbamazapine, Valproic Acid</td>
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Drug-Induced Neutropenia

- Idiosyncratic drug reaction leading to profound neutropenia or agranulocytosis
- Pathogenesis poorly understood, and studies are difficult because it is rare, sporadic, and transient.
  - Anti-neutrophil antibodies
    - Autoantibodies
    - Drug-dependent antibodies
    - Complement binding in some cases
    - Graves’ disease: antibodies that cross-react with TSH

Unlike chronic neutropenia, DIN is associated with significant morbidity and a mortality of 10%
A 31-year-old woman referred for neutropenia

HPI:
- Age 16: Episodic abdominal pain, fever, and vomiting
  - After multiple episodes: dx appendicitis
  - Symptoms resolved after appendectomy
- Post-operatively, WBC fell to 2000 with an ANC of 500
  Neutropenia has persisted ever since

PMH:
- In retrospect: frequent upper respiratory illnesses as a child, including several episodes of pneumonia
- 1 year ago: begun on weekly G-CSF

ROS: LUQ pain, nausea and vomiting for 1-2 days after taking G-CSF
Chronic Idiopathic Neutropenia

- Chronic neutropenia termed “Non-Immune Chronic Idiopathic Neutropenia in Adults (NI-CINA)”
- Normal marrow cytogenetics; variable cellularity (hypocellular/hypercellular)
- No evidence of autoimmune disease, nutritional deficiency, myelodysplasia
- Benign clinical course, often diagnosed on routine blood tests in asymptomatic patients

Pathophysiology

- NO IDEA!!!
- Majority remain totally unexplained
Management of the Neutropenic Patient

**Diagnostic**
- Stop potential offending drugs
- Bone marrow aspiration/biopsy
- Serologic studies: ANA, viral titers, anti-neutrophil antibodies
- R/O Primary malignancy:
  - Chromosome analysis
  - Sucrose-hemolysis test; flow cytometry
Management of the Neutropenic Patient

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**Therapeutic**
- Aggressive treatment of infections
- Immune neutropenia: steroids, IgG
- LGL: low dose MTX
- G-CSF: SCN, CH, recovery from drugs
- Stem cell transplant: SCN
Treatment of Neutropenia: G-CSF or NO?

- Responses to G-CSF documented in neonatal, primary and secondary immune, and NI-CINA
  - Treatment is frequently unnecessary
  - Reserved for recurrent or serious infections
  - May cause flare of joint disease in setting of RA

- Shortens the time to neutrophil recovery in drug-induced neutropenia/agranulocytosis
  - Evidence-based data lacking: only randomized trial had only 24 patients, and used a subtherapeutic dose of G-CSF
  - Meta-analyses & retrospective analyses suggest shorter time to WBC recovery, reduced cost, ? reduced mortality
  - 10% mortality rate, safety and efficacy justify G-CSF use in this setting
CASE PRESENTATION

5 yo boy w/ sinusitis not responding well to antibiotics

HPI:
- 3 days PTA: fever, cheek pain
- Xray: opacification of R maxillary sinus
- Begun on oral antibiotics; admitted for poor response

PMH:
- Multiple episodes of otitis media in first two years of life, requiring tube placement
- S/p two episodes of pneumonia requiring hospitalization
- S. aureus abscess of the thigh at age 3

CBC:
- WBC 22,000, 88% polys, 5% bands
- Plts 608K
- Hb 9.9
Mechanisms of Neutrophil Function

Receptor function/chemotaxis/phagocytosis
- Leukocyte adhesion deficiency
- Hyper IgE syndrome (Job’s syndrome)
- Chediak-Higashi syndrome

Degranulation
- Specific granule deficiency

Oxygen-dependent killing
- Chronic granulomatous disease
- Neutrophil G6PD deficiency
- Glutathione reductase/synthase deficiency

Oxygen-independent killing
- Specific granule deficiency
- Myeloperoxidase deficiency
Chronic Granulomatous Disease

**Etiology:**
- Failure of the respiratory burst
- Decreased activity of NADPH oxidase
- Heterogeneous disorder
  - X-linked (gp91 phox)
  - Others autosomal recessive (67,41,22)

**Clinical manifestations:**
- Chronic recurrent infections
- Onset early in life
- Usual organisms: nonencapsulated bacteria (S. aureus, E. coli, other gnrs); fungi (candida, aspergillus)
Chronic Granulomatous Disease

**Treatment**

- IV antibiotics for infections
- Interferon gamma.
  - Multicenter trial of IFN showed 70% reduction in infections *despite* failure to demonstrate increased production of O₂
- Stem cell transplantation
- Gene therapy
  - Clinical trials of transplantation of transduced autologous CD34+ cells without marrow conditioning. Patients show low-level engraftment that decreases over time. Two patients showed long-term reconstitution with insertion in proto-oncogene loci; both subsequently developed MDS
3-year-old boy with fever, sore throat, poor response to abx

HPI:
- PTA: sore throat and high spiking fever despite antibiotics
- 10 days later, diffuse adenopathy and hepatosplenomegaly
- Cervical lymph node bx: malignant lymphoma
- Spontaneous remission over next 3 months
- 1 year later, recurrent adenopathy responsive to steroids
- Subsequent relapse with adenopathy, respiratory distress, and death
- Autopsy: infiltration of lung, liver, nodes, spleen, kidneys with immature lymphoid cells and histiocytes

PMH:
- Recurrent ulcerations of buccal mucosa from early age
- Light coloring, photophobia, nystagmus

FH: Sister with photophobia, nystagmus

Adapted from Blood 20:330, 1962
Chediak-Higashi Syndrome

Lazarchick, J. et al. ASH Image Bank 2005:101296
Chediak-Higashi Syndrome

**Etiology:**
- generalized defect of granule morphogenesis
- Neutrophils show multiple functional abnormalities, including impaired granule release
- Defects in the “LYST” gene, which appears involved in membrane fusion events and granule trafficking

**Clinical manifestations:**
- oculocutaneous albinism.
- Recurrent bacterial infections, neuropathies
- accelerated phase: hepatosplenomegaly, pancytopenia, and death, perhaps 2o EBV c/w hemophagocytic lymphohistiocytosis
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Functional neutrophil disorders are rare, but provide important insights into normal neutrophil biology.