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Leukocytosis and Neutropenia

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I have no financial relationships to disclose.

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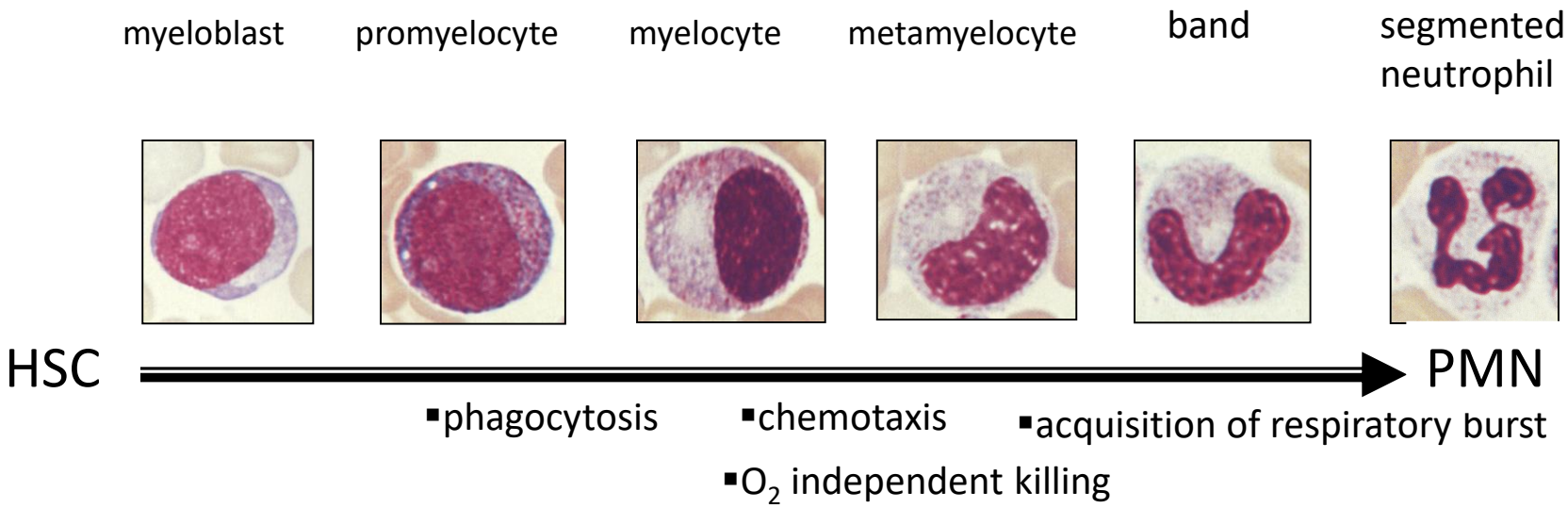
I will **NOT** include discussion of off-label or investigational use of any products in my presentation.

MYELOPOIESIS



Role of G-CSF:

- Proliferation of myeloid progenitors
- Induction of myeloid maturation
- Protection from apoptosis
- Enhancement of neutrophil function

Life Span of a Neutrophil

- Maturation in the bone marrow: 7-10 days
- Circulation in the peripheral blood: 3-24 hours
- Duration in the tissues: 2-3 days

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.

CASE PRESENTATION

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
- Post-splenectomy
- Non-hematologic malignancy
- Marrow stimulation (ITP, hemolysis, CMT)

PRIMARY HEMATOLOGIC DISEASE

- CML
- Other MPD

EVALUATION OF ADULT W/ LEUKOCYTOSIS

Neutophilia is usually reactive, indicative of a normal functioning bone marrow. Bone marrow evaluation is often unnecessary

- Repeat WBC to R/O factitious or artifactual elevation
- Evaluation for acute/chronic infection or inflammation
- LAP score-of limited value in era of 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

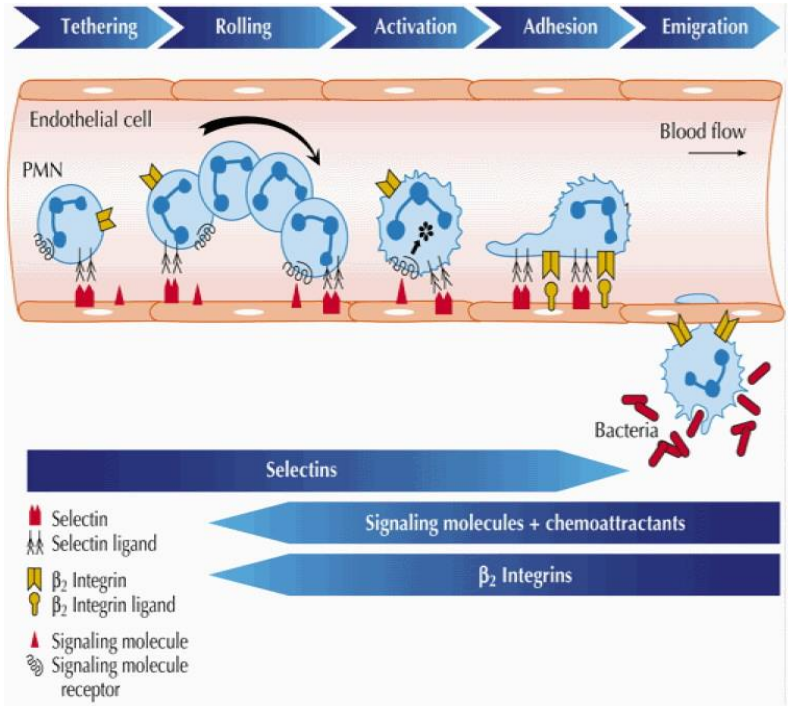
1 month old infant with delayed umbilical cord separation
High grade fever, MRSA infection, and WBC of 90,000
Poorly healing skin lesions, otitis, failure to thrive
Poor response to antibiotics
What to do??

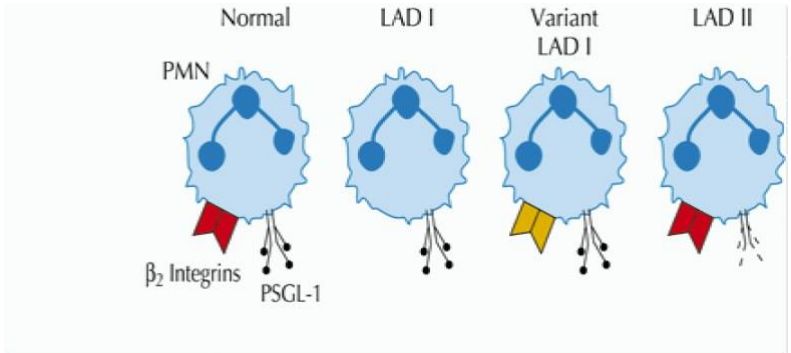
Adapted from Pediatr Transplantation 11:453-5, 2007

LEUKOCYTOSIS: DIFFERENTIAL DIAGNOSIS

Congenital
- Hereditary neutrophilia
- Down's sx
- Leukocyte Adhesion Deficiency

ADHESION MOLECULES AND LAD





β2 Integrins	Present	↓, Absent	Defective	Normal
Selectin ligand (PSGL-1)	Present	Normal	Normal	Defective

Pathogenesis:

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

Curr Opin Hematol; 9: 30-35, 2002

LEUKOCYTE ADHESION DEFICIENCY

Clinical manifestations:

- elevated WBC
- recurrent infections, mainly cutaneous abscesses, gingivitis
- Many die before age 2

Treatment:

- Stem cell transplant: treatment of choice (performed on the patient described here)

NEUTROPENIA

ANC	Clinical Significance
1000-1500	Statistically abnormal but not clinically significant
500-1000	Slight predisposition to infection
200-500	Significant predisposition to infection; IV antibiotics for febrile illness
<200	Very high risk of infection; decreased signs of inflammation; aggressive in-patient treatment for febrile illness

In chronic neutropenia, patients frequently have little or no manifestations of neutropenia with counts of 50-100

CASE PRESENTATION

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 6 months despite antibiotics
- with widespread infection, boils, thrush

Adapted from Kostmann, Acta Paediatr Scand 1956

CONGENITAL NEUTROPENIA		
Syndrome	**Inheritance**	**Gene (s)**
~~Ethnic neutropenia~~ Duffy-null associated neutrophil count (DANC)	?	DARC SNPS
Benign familial neutropenia	AD	Unknown
Severe congenital neutropenia	AD	ELANE (55-60%)
	AR	HAX1 and G6PC3 (<5%)
	X-linked	WASP (<5%)
	Unknown	Unknown (40%)
Cyclic neutropenia	AD	ELANE (95-100%)
Immunodeficiency Syndromes Associated with Neutropenia		
Schwachman-Diamond Syndrome	AR	SBDS (100%)
Fanconi Anemia	AR and X-linked	FANC A-P genes
Dyskeratosis Congenita	X-linked	DKC1 (80%)
	AD	TERC (0-20%)
	AR	TERT (0-20%)
Glycogen storage disease Ib	AR	SLC37A4 (100%)
Myelokathexis	AD	CXCR4 (100%)
Chediak-Higashi syndrome	AR	LYST (100%)
Griscelli syndrome II	AR	RAB27A (100%)
Hermansky-Pudlak syndrome II	AR	AP3B1 (100%)
Cartilage-hair hypoplasia	AR	RMRP (100%)

SEVERE CONGENITAL NEUTROPENIA

Congenital agranulocytosis

- rare
- autosomal dominant, recessive, and sporadic cases
- severe infections; survival dramatically changed by treatment with G-CSF
- high incidence (30% over 10 years) of MDS/AML

SEVERE CONGENITAL NEUTROPENIA

Autosomal dominant form of SCN:

- linked to mutations in the neutrophil elastase (ELANE)
- Mutant elastase accumulates in the cytoplasm, and activates the "unfolded protein response," a cellular stress response that results in apoptosis.

Autosomal recessive SCN:

- Kostmann's Syndrome: original syndrome of 50 years ago
- Linked to mutations in HAX1, a mitochondrial protein
- Disruption of HAX1 in myeloid cells destabilizes the mitochondrial membrane and leads to apoptosis

AML associated with a truncation mutation of the G-CSF receptor of uncertain pathogenetic significance

CYCLIC NEUTROPENIA

- dominantly inherited syndrome w/ cycle of neutropenia q 15-35 days
- marrow during neutropenia: myelocyte arrest
- Usually benign; patients with severe infections may respond to G-CSF
- Virtually 100% of patients have mutations in ELANE
- 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:

Hydroxychloroquine, ASA 81mg, Prednisone 5; recent taper from 50mg

EXAM: Malar rash; no active joint disease

LABS: WBC 1.8

AUTOIMMUNE NEUTROPENIA

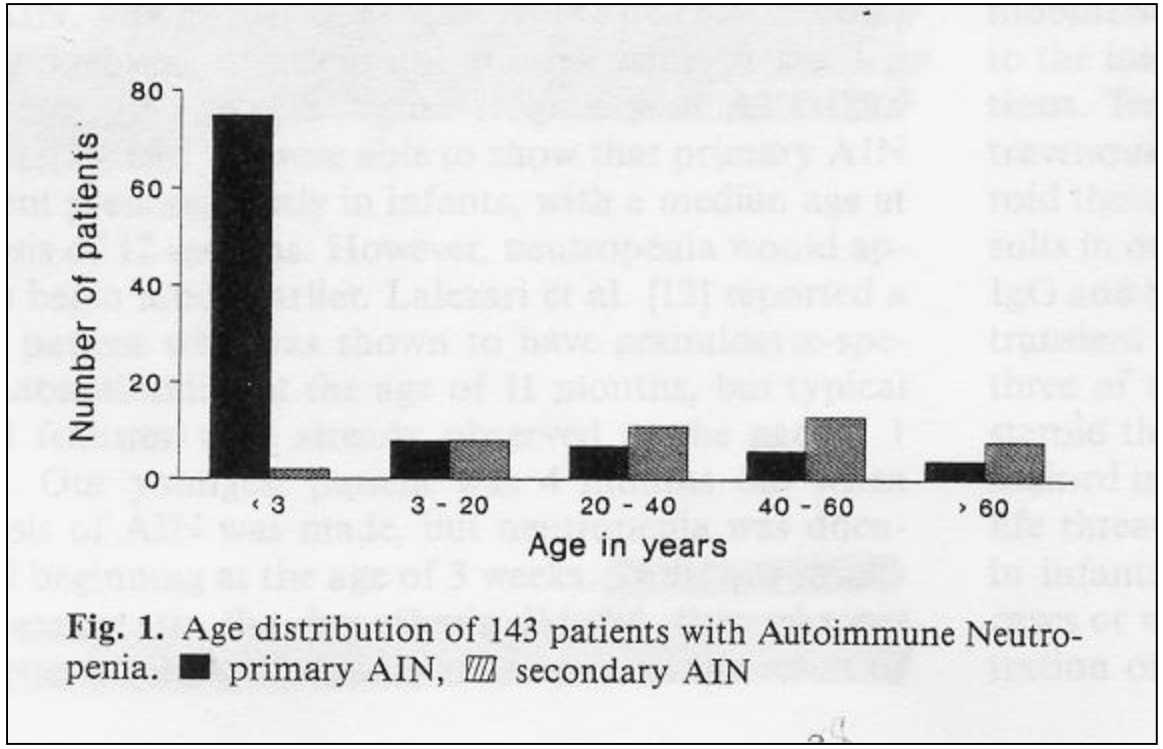


Fig. 1. Age distribution of 143 patients with Autoimmune Neutropenia. ■ primary AIN, ▨ secondary AIN

Primary AIN:

- Seen primarily in children
- Associated with abs against common ags

Secondary AIN

- Seen primarily in adults
- Associated with AID
- Associated with LGL
- Associated (rare) with leukemia/lymphoma

Ann Hematol 63: 249-252, 1991

PRIMARY AUTOIMMUNE NEUTROPENIA

A disease of childhood caused by a-neutrophil antibodies

- 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 infection

ANTIGEN	PREVIOUS NOMENCLATURE	GLYCOPROTEIN
HNA-1a	NA1	FcγIIIb (CD16)
HNA-1b	NA2	FcγIIIb (CD16)
HNA-1c	SH, NA3	FcγIIIb (CD16)
HNA-2a	NB1	CD177(gp50-64)
HNA-3a	5b	Gp70-95
HNA-4a	MART	CD11a
HNA-5a	OND	CD11b

2^0 AUTOIMMUNE NEUTROPENIA

- Associated with autoimmune disease
- Most commonly seen in adults
- Most common association: SLE, RA

Associated diseases in 42 patients with 2^0 AIN

Underlying disease	**Patients (n)**
Autoimmune thrombocytopenia	12
Evans' syndrome	3
Autoimmune hemolytic anemia	1
Systemic lupus erythematosus	9
Rheumatoid arthritis	2
Felty's syndrome	2
Leukemia	3
Non-Hodgkin's lymphoma	3

Adapted from Ann Hematol 63: 249-252, 1991

AUTOIMMUNE NEUTROPENIA IN SLE

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

Pathophysiology:

- Neutrophil-specific antibodies
- Immune-complex mediated destruction
- Increased apoptosis of neutrophils
- Decreased marrow neutrophil production

CASE PRESENTATION

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

LGL-associated neutropenia

- Shares many features with Felty's syndrome
- Monoclonal neoplastic disorder, while Felty's traditionally is polyclonal

Both have a very high (90%) incidence of HLADR4, suggesting they are a spectrum of the same disease

PITFALLS OF ANTI-NEUTROPHIL AB TEST

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

Effect on outcome remains undefined

- no “gold standard”
- non-neutropenic populations often have detectable antibody
- poor correlation between level of antibody and degree of neutropenia

WHEN DO I CHECK ANTI-WBC ABS IN ADULTS w/ NEUTROPENIA?

NEVER

CASE PRESENTATION

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

Anti-thyroid medications	Carbamizole Methimazole Thiouracil
Antibiotics	Cephalosporins Penicillins Sulfonamides Chloramphenicol
Anticonvulsants	Carbamazapine Valproic Acid

DRUG-INDUCED NEUTROPENIA

- Idiosyncratic drug reaction leading to profound neutropenia or agranulocytosis
- Pathogenesis poorly understood, and studies are difficult 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%

CASE PRESENTATION

A 31-year-old woman referred for neutropenia

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 1-2 days after taking G-CSF

NON-IMMUNE CHRONIC NEUTROPENIA

Chronic neutropenia

- Normal marrow cytogenetics; variable cellularity
- No evidence of autoimmune disease, nutritional deficiency, myelodysplasia
- Benign clinical course, often diagnosed on routine blood tests in asymptomatic patients
- Variable need for cytokine support

Pathophysiology

- NO IDEA!! Probably a heterogeneous disorder.
- Patients with myeloid hypoplasia: selective decrease in CD34+/CD33- progenitors (Papadaki Blood 101:2591, 2003) and increased production of TNF (Papadaki AJH 65:271, 2000)

MANAGEMENT OF THE NEUTROPENIC PATIENT

Diagnostic
- Stop potential offending drugs
- Bone marrow aspiration/biopsy
- Serologic studies: ANA, viral titers, (a-neutrophil abs)
- R/O Primary malignancy:
  - Chromosome analysis
  - Flow cytometry for PNH

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 chronic idiopathic neutropenia (CIN)

- 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:

- 3days 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

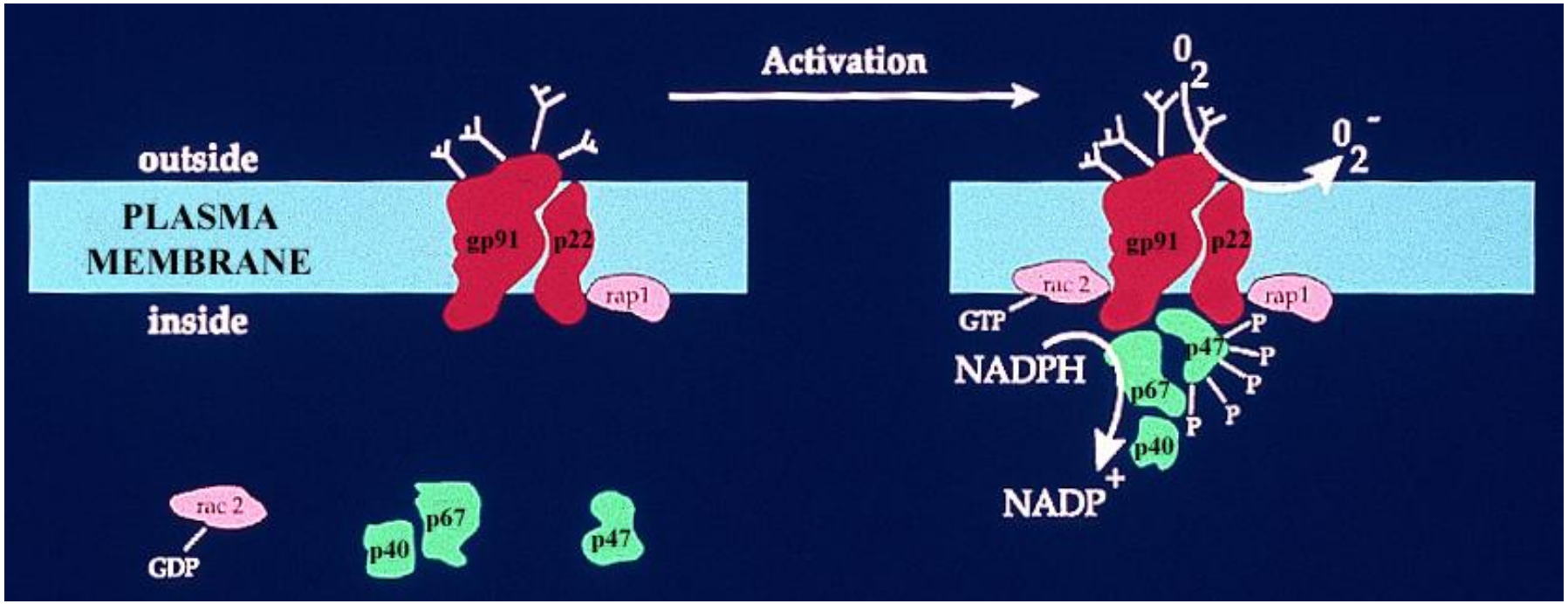
Receptor function/chemotaxis/phagocytosis
- Leukocyte adhesion deficiency
- Hyper IgE syndrome (Job's syndrome)

Degranulation
- Chediak-Higashi syndrome
- Specific granule deficiency

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

Oxygen-independent killing
- Specific granule deficiency
- Myeloperoxidase deficiency

PHAGOCYTE NADPH OXIDASE (NOX2)



Etiology:

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

CHRONIC GRANULOMATOUS DISEASE

Recurrent infections, with onset usually (not always!) early in life.
Oldest patient at diagnosis: age 69

Single infections with opportunistic organisms

Chronic inflammation:

- Granulomatous colitis
- Restrictive lung disease
- Obstruction of gastric outlet, ureters

Immune-mediated disease

- Discoid lupus
- Macrophage activation syndrome / hemophagocytic lymphohistiocytosis

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 O_2
- 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

“TAKE-HOME MESSAGES”

- Leukocytosis is usually reactive and the sign of a healthy bone marrow responding to external signals.
- Peripheral smear and PCR findings can usually provide clues to the less common primary marrow disorders associated with elevated neutrophils. Don‘t miss CML!
- Neutropenia is more commonly a manifestation of a primary marrow problem, although autoimmune disease and sequestration both occur.
- Although rare, study of congenital neutropenia has provided key insights into neutrophil biology and AML.
- Functional neutrophil disorders are rare, but provide important insights into normal neutrophil biology.

REVIEW QUESTION 1

A patient with HIV and a low CD4 count is begun on trimethoprim-sulfamethoxazole. Six weeks later he comes to the emergency room with a fever of 102°F and a tonsillar exudate. CBC shows a WBC of 1500 with 5% neutrophils, a normal H/H and platelet count. Antibiotic prophylaxis is D/C'd. Appropriate therapy should include:

A. Start the patient on amoxicillin and schedule him to return to clinic the following day for follow-up of his throat culture.
B. Start the patient on amoxicillin and steroids and admit for observation.
C. Start the patient on amoxicillin and admit for observation.
D. Admit the patient to the hospital and begin on broad-spectrum antibiotics.
E. Admit the patient to the hospital and begin broad-spectrum antibiotics and G-CSF.

REVIEW QUESTION 1

A. Start the patient on amoxicillin and schedule him to return to clinic the following day for follow-up of his throat culture.
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E. Admit the patient to the hospital and begin broad-spectrum antibiotics and G-CSF.

With otherwise normal or stable counts, acute profound neutropenia is essentially always drug-induced. This is true even in HIV, despite the many possible etiologies for neutropenia. This agranulocytosis is probably induced by trimethoprim-sulfa. With an absolute neutrophil count of 75, the patient needs emergent broad-spectrum antibiotics. WBC recovery can be speeded by the administration of G-CSF, and G-CSF is recommended.

REVIEW QUESTION 2

A 55 year old man presents with substernal chest pain and is diagnosed with acute myocardial infarction. Admission CBC reveals a WBC of 16,000, Hct 40, and Platelets of 280K. What is the most likely explanation for his leukocytosis:

A. Acute bacterial infection causing coronary ischemia
B. Undiagnosed chronic myelogenous leukemia
C. Stress-induced demargination of neutrophils
D. Cytokine release stimulating increased marrow production and release of neutrophils
E. Drug induced neutrophilia

REVIEW QUESTION 2

A. Acute bacterial infection causing coronary ischemia
B. Undiagnosed chronic myelogenous leukemia
C. Stress-induced demargination of neutrophils
D. Cytokine release stimulating increased marrow production and release of neutrophils
E. Drug induced neutrophilia

Acute myocardial infarction is commonly associated with leukocytosis and patients often also present with low grade fever. Acute leukocytosis in almost any setting occurs by demargination, in this case in response to the release of catecholamines during the stress of the cardiac ischemia. Cytokine induced marrow proliferation and release of neutrophils reflect more chronic inflammation, and might occur if, for example, the patient went on to develop post-infarction pericarditis. There is no reason to consider a chronic myeloproliferative disease or sepsis in the absence of other evidence for a primary hematologic condition.

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