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laboratory medicine.

## PEARLS OF LABORATORY MEDICINE

### **Neutrophil Defects**

*Vijaya Knight, MD, PhD, (D)ABMLI*

*National Jewish Health Advanced Diagnostic Laboratories*

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# Neutrophils: Background

- Neutrophils or polymorphonuclear leukocytes (PMN) are bone-marrow derived, terminally differentiated cells of the innate immune system
- Most abundant cells in peripheral blood;  $\sim 10^9$  produced per hour
- Short lifespan of 5-7 days in circulation
- Characterized by segmented nucleus, primary, secondary, tertiary, and secretory cytoplasmic granules
- First line of immune defense against bacterial and fungal infections



# Neutrophil Defects

- 5% of primary immune deficiencies are attributed to neutrophil defects
- Increased frequency of bacterial and fungal infections at epithelial sites (skin and gastrointestinal tract)
- Increased frequency of disseminated infections
- Infections tend to be very aggressive
- Management includes bone marrow transplant or lifelong prophylaxis with antibiotics



# Neutrophil Defects

Defects of development manifest as neutropenia:

- Intrinsic or Genetic
- Extrinsic or Acquired

Defects of function manifest as defective adherence, motility, oxidative burst, or extracellular mechanisms:

- Generally intrinsic, due to defined genetic mutations
- Extrinsic causes less well-defined



# Examples of Intrinsic Defects

DISORDER	INHERITANCE	GENETIC DEFECT	PHENOTYPIC DEFECT
Severe congenital neutropenia	AD, AR, sporadic	ELANE, HAX1, GF11 mutation	Severe neutropenia, arrest at promyelocytic stage
Shwachman-Diamond syndrome	AR	SDBS mutations	Moderate to severe neutropenia, anemia, growth retardation, pancreatic insufficiency
Specific granule deficiency	AR	CEBPE mutation	Absent secondary and tertiary granules, primary granules lack defensins
Griscelli syndrome	AR	RAP27A mutation	Neutropenia, frequent pyogenic infections
Chediak-Higashi syndrome	AR	LYST mutation	Mild neutropenia, recurrent infections, defective bacterial killing, enlarged, irregular azurophilic granules

# Acquired Neutropenia

## 1. Decreased output:

- Drug-induced bone marrow suppression leading to decreased output (e.g.: Cyclophosphamide)

## 2. Increased destruction:

- Alloimmune: transplacental passage of IgG antibodies to neutrophil-specific antigens
- Autoimmune: anti-neutrophil antibodies in ALPS or ITP

## 3. Sequestration without destruction:

- Splenomegaly (e.g.: malaria, portal hypertension)

# Mediators of Leukocyte Adhesion

- Migration of neutrophils to tissues depends on bidirectional interactions between  $\beta$  integrins and selectins
- Sialyl Lewis<sup>x</sup>, a fucosylated receptor, mediates initial tethering to the endothelium through low affinity interactions
- High affinity interactions between CD11a, CD11b, CD11, CD18 and selectins strengthen adhesion
- Kindlin3 mediates further activation of  $\beta$  integrins through chemokine mediated signals from within the cell



# Comparison of LAD I, II, and III

	LAD I	LAD II	LAD III
Affected gene	<i>ITGB2</i>	<i>FUCT1</i>	<i>FERMT3</i>
Molecule involved	CD18	Sialyl Lewis <sup>x</sup>	KINDLIN3
Recurrent infections	+++	+	+++
Leukocytosis	+	+	++
Delayed umbilical cord separation	+++	-	+++; bleeding
CD18 expression	Absent	Normal	Normal
SLex (CD15a)	Normal	Absent	Normal
Treatment	BMT if severe	Fucose administration	BMT

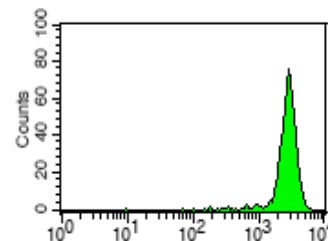
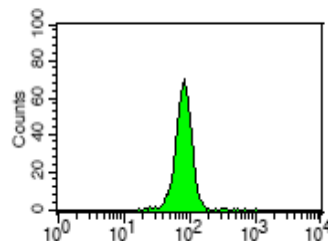
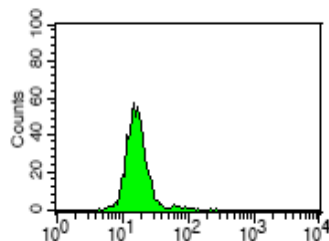
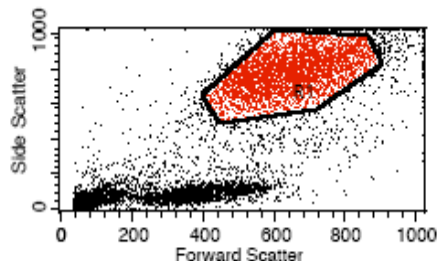


# Diagnosis of LAD I

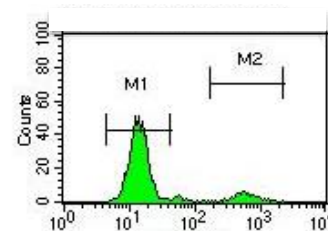
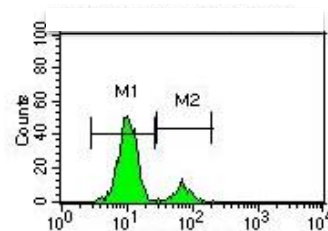
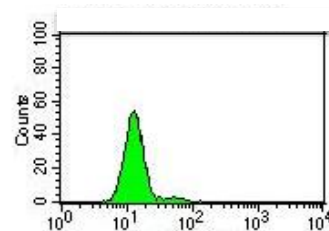
- **LAD I:** Flow cytometry analysis of surface expression of the integrins: CD11a, b, and c, and CD18
- **LAD II:** Analysis of CD15s surface expression by flow cytometry
- **LAD III:** Genetic analysis for *FERMT3* mutations that result in defective Kindlin-3 protein expression



# Expression of CD18 and CD11a Post Bone Marrow Transplant



Healthy control



Patient post BMT

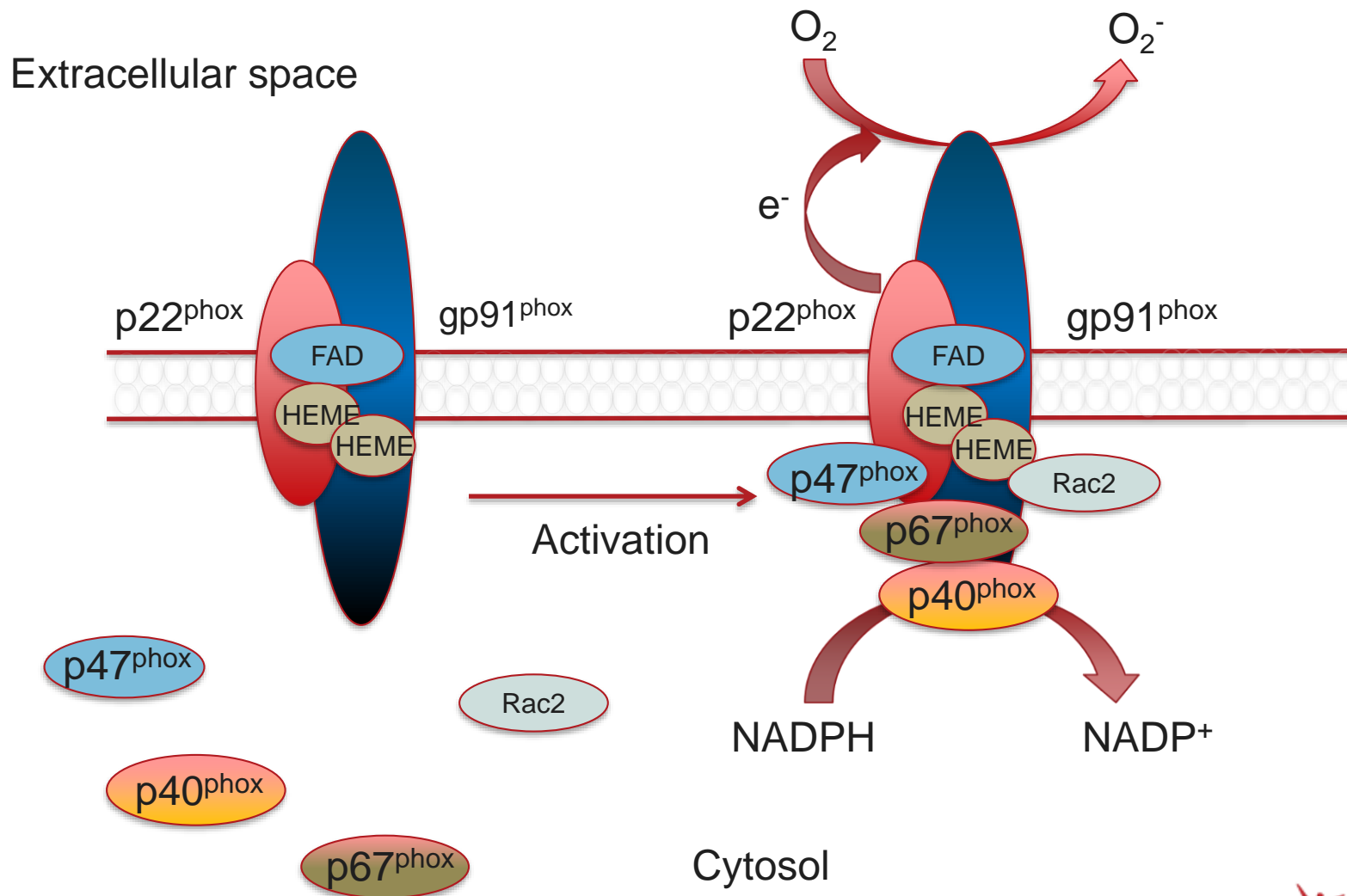
Isotype control

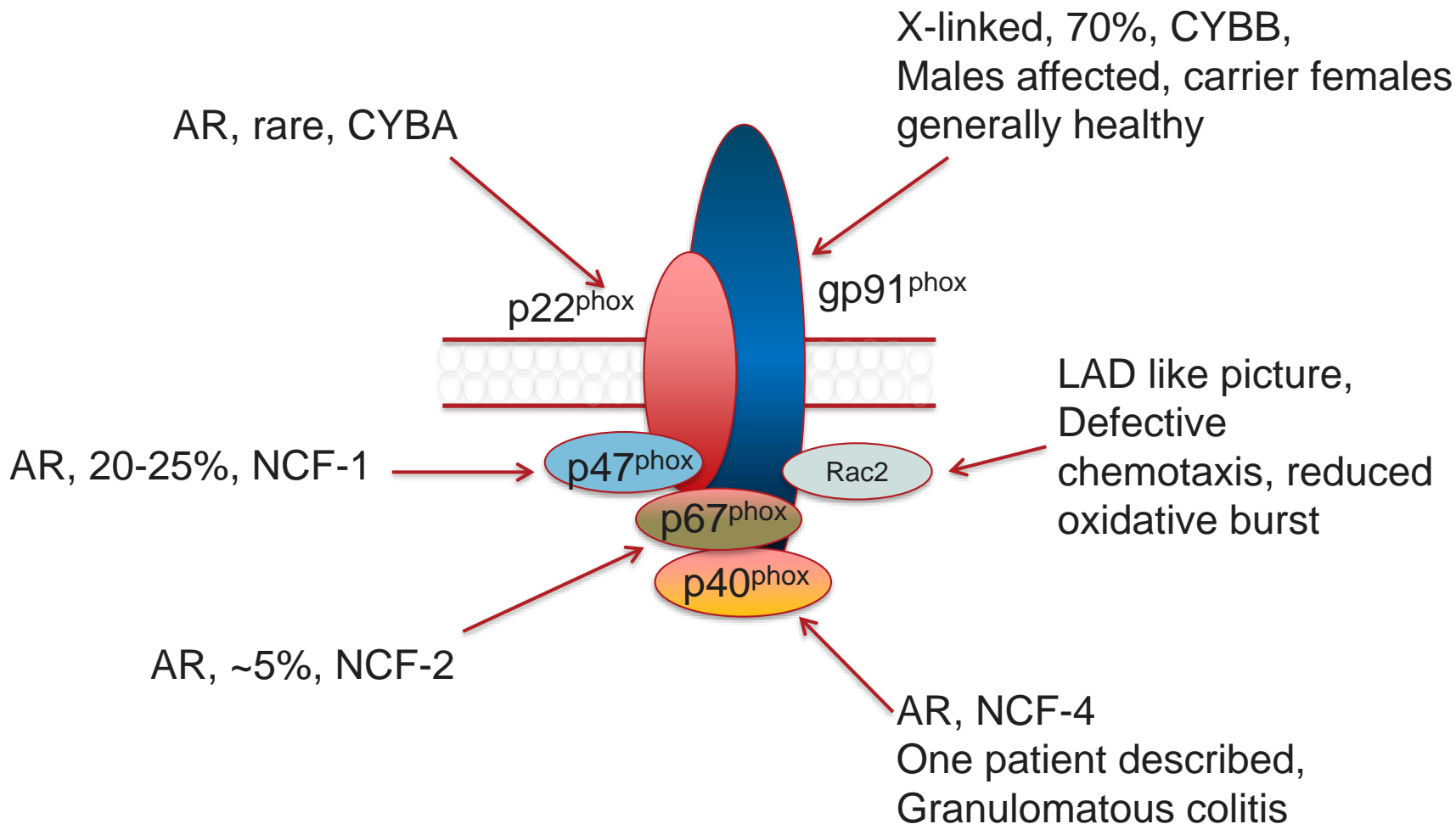
CD11a

CD18

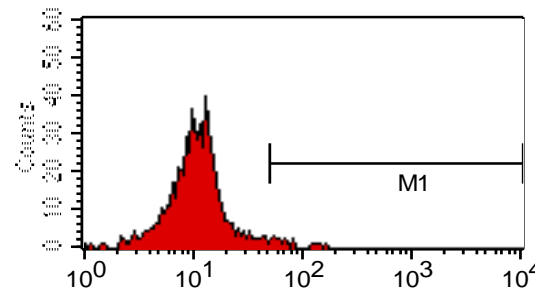
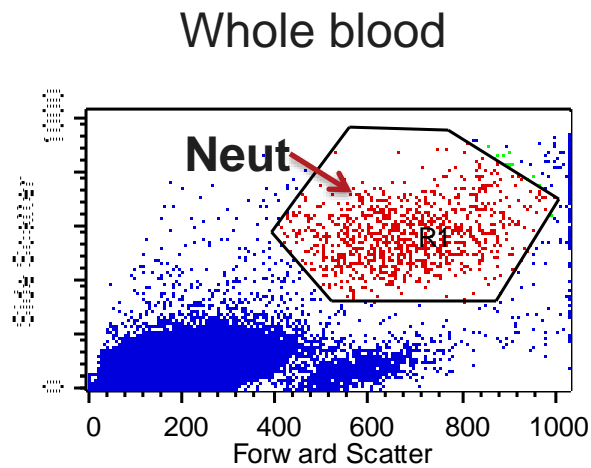


# The NADPH Complex

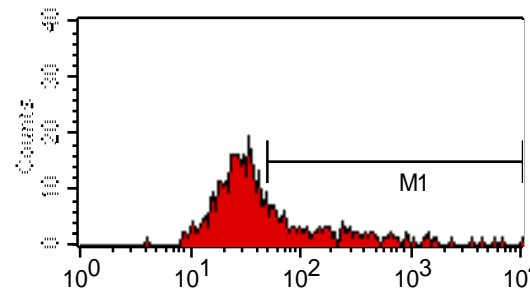




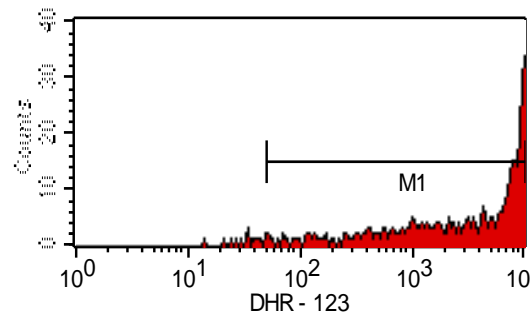
# Dihydrorhodamine 123 Assay for Neutrophil Oxidative Burst



Unstained Neutrophils



Neutrophils + DHR 123

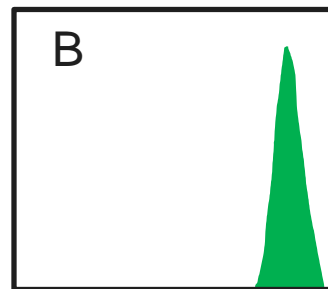
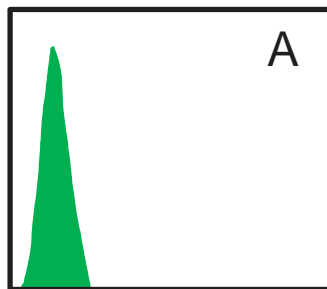


Neutrophils + DHR123 + PMA



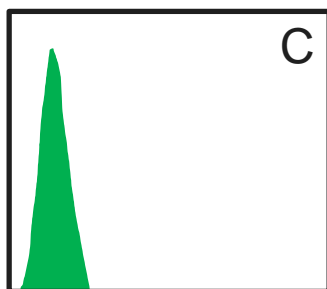
# Flow Cytometry in CGD

Normal neutrophils

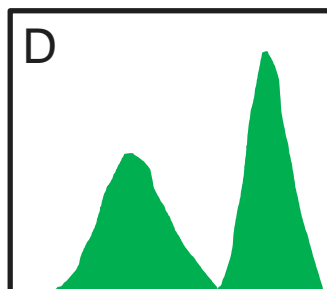


Unstimulated cells

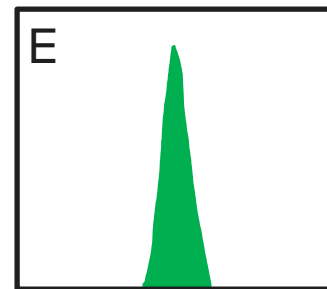
Stimulated cells



CGD patient



CGD carrier



AR variant

→ Stimulated cells



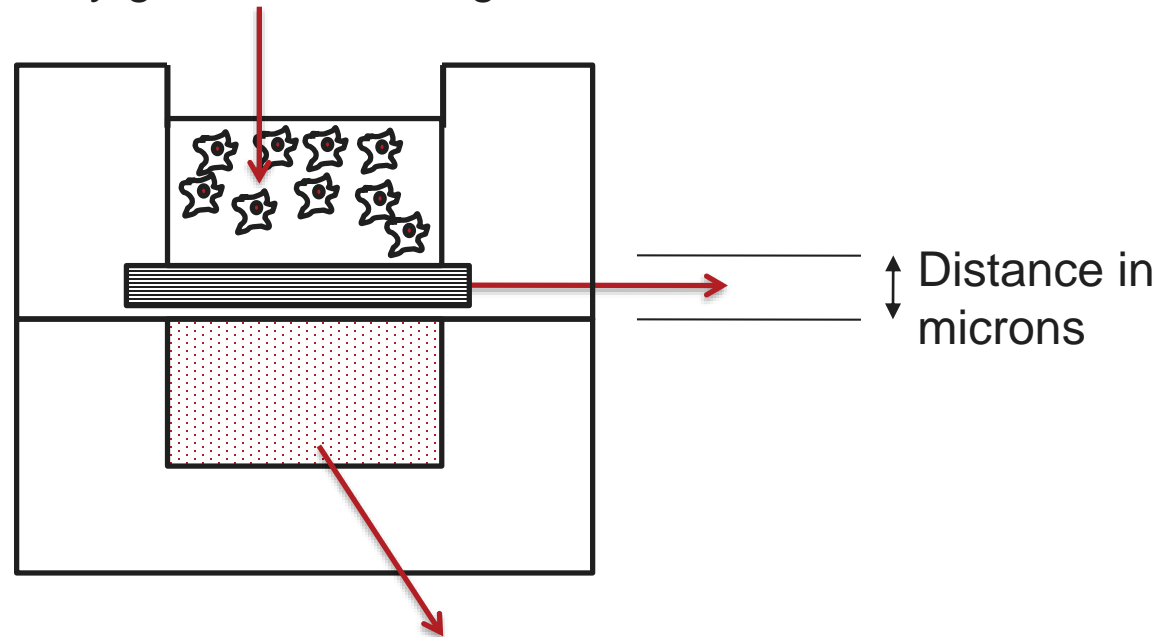
# Neutrophil Chemotaxis Defects

- Rarely isolated defects
- Rac2 associated with chemotaxis and oxidative burst defects
- Other defects include SCN4, Papillon Lefevre syndrome, Schwachman-Diamond syndrome, Down syndrome or trisomy 21, Chediak-Higashi syndrome, among others



# Assessing Neutrophil Chemotaxis

Neutrophils isolated from peripheral blood  
through density gradient centrifugation



Chemoattractant (zymosan  
activated serum, results in  
formation of C5a)

# Summary

1. Defects of neutrophil function encompass both inherited and acquired factors
2. Neutrophil defects generally manifest as increased susceptibility to bacterial and fungal infections
3. Neutrophil function is assessed by fairly simple laboratory tests
4. Neutrophil defects may be part of complex syndromes with diverse presentations



# References

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# Disclosures/Potential Conflicts of Interest

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