

**Lecture # 4**  
**Innate immunity (Cellular defense mechanism)**

At the end of this lecture, students will be able to

1- Definition of Phagocytosis

2- Steps of Phagocytosis

3- Cytotoxicity of NK cells

Phagocytosis performed by phagocytic cells ( MO + Monocytes + neutrophils)

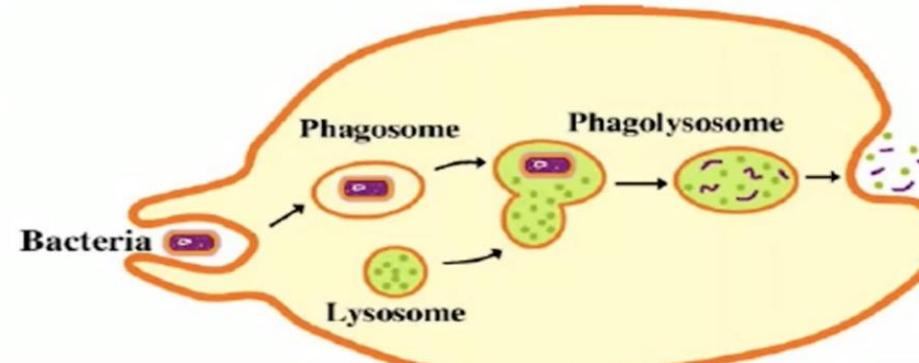
When phagocytosis occurs?

It occurs at the site of bacterial invasion

## I) Definition of Phagocytosis

**Phago-cyt-osis**  
**Phago= eating Cyte= cell Osis= Process**

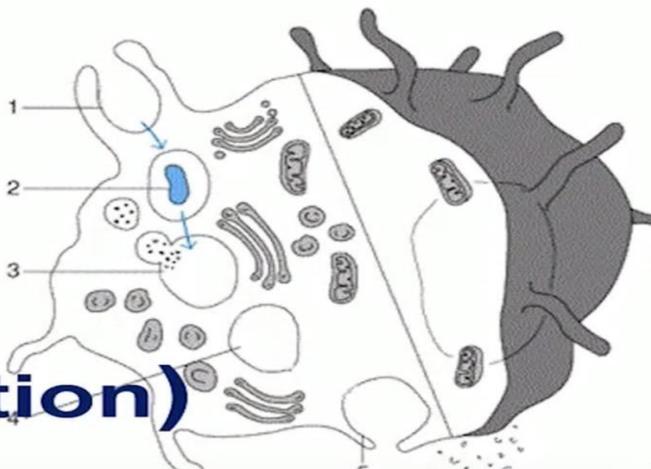
**It's a process of destroying a particle by cells**



**(Cell eating)**

## II) Steps of phagocytosis

- 1) Chemotaxis
- 2) Migration
- 3) Attachment
- 4) Ingestion
- 5) Killing (Digestion)

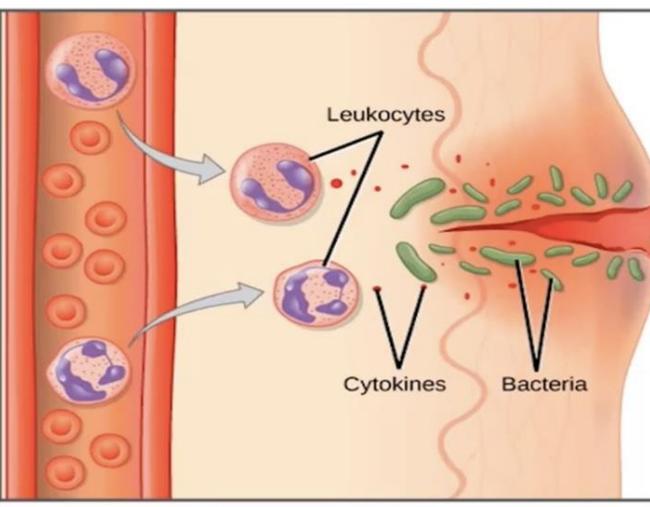


### 1) Chemotaxis

Chemotactic factors



Bacterial endotoxin  
C5a  
IL-8



2- Migration

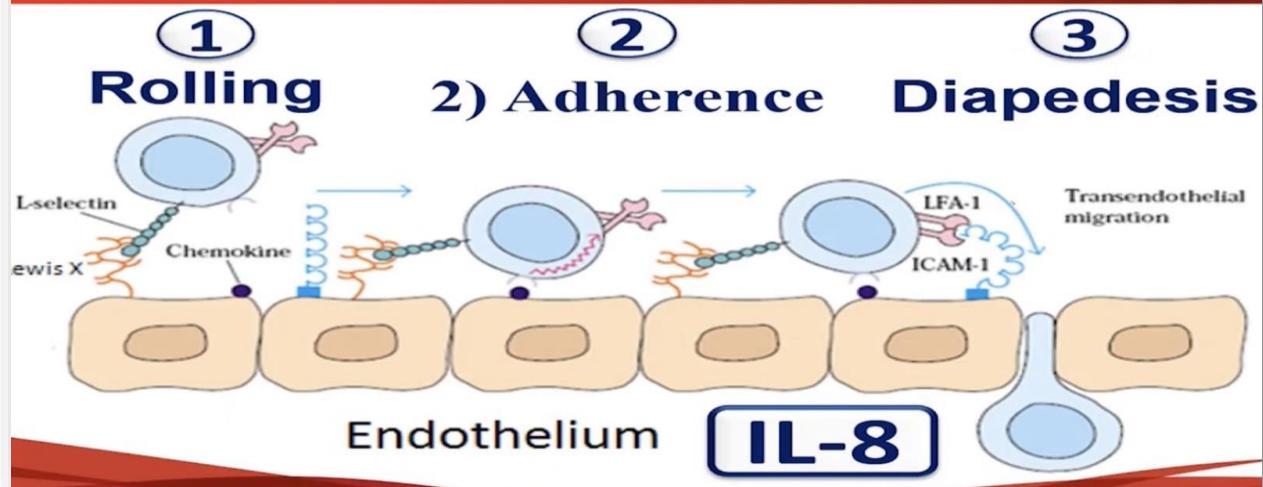
A- Rolling

B- Adherence (integration)

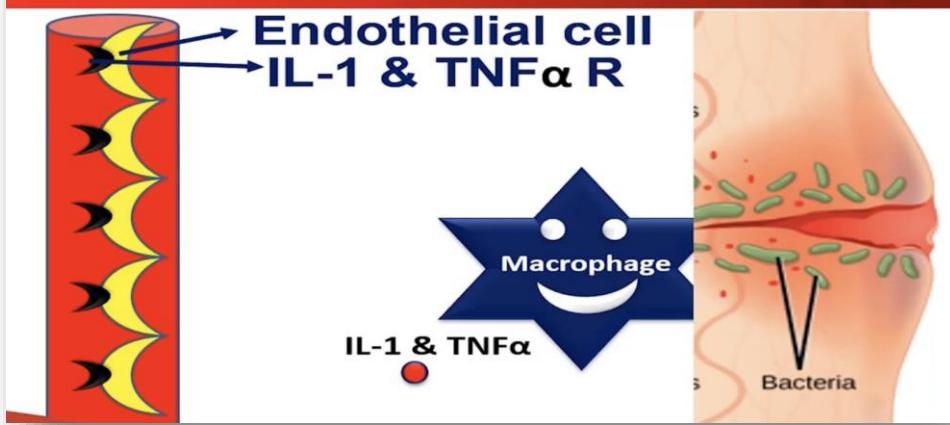
C-Diapedesis or extravasation

A- Rolling

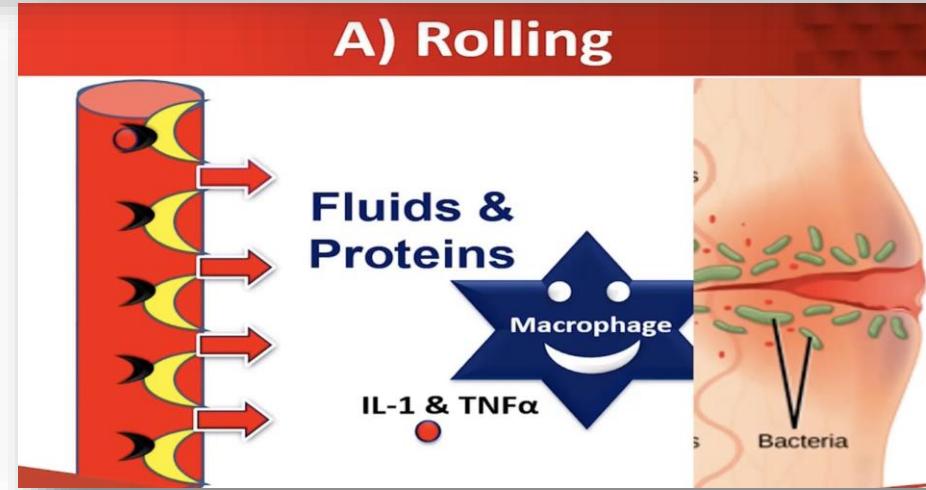
## B) Adherence (Stop movement)



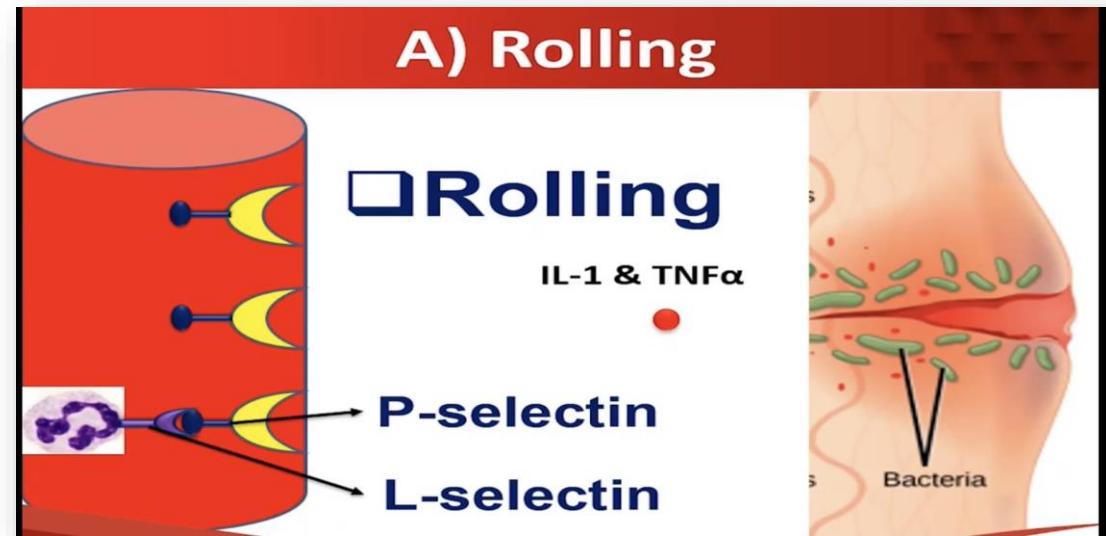
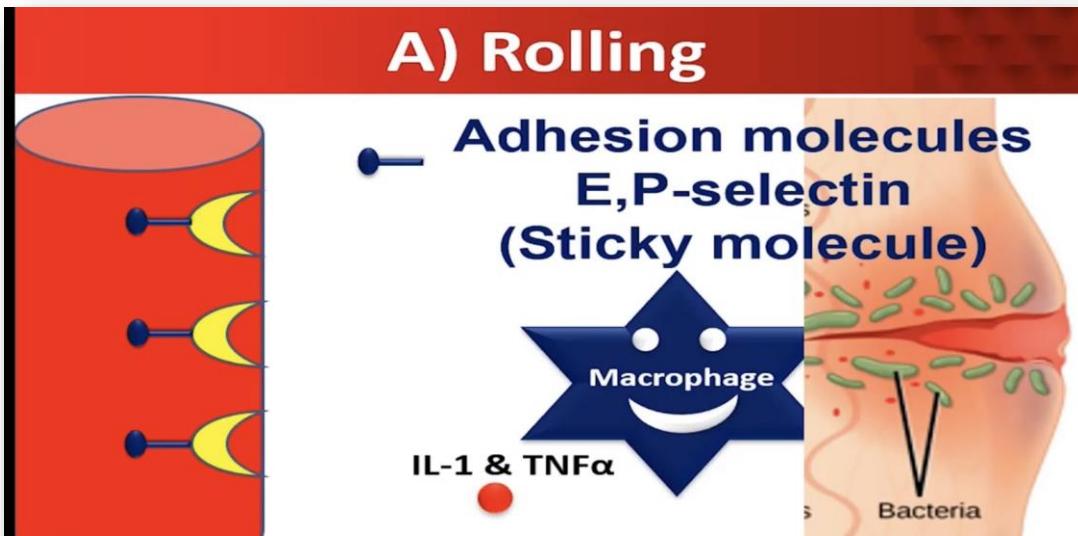
## A) Rolling



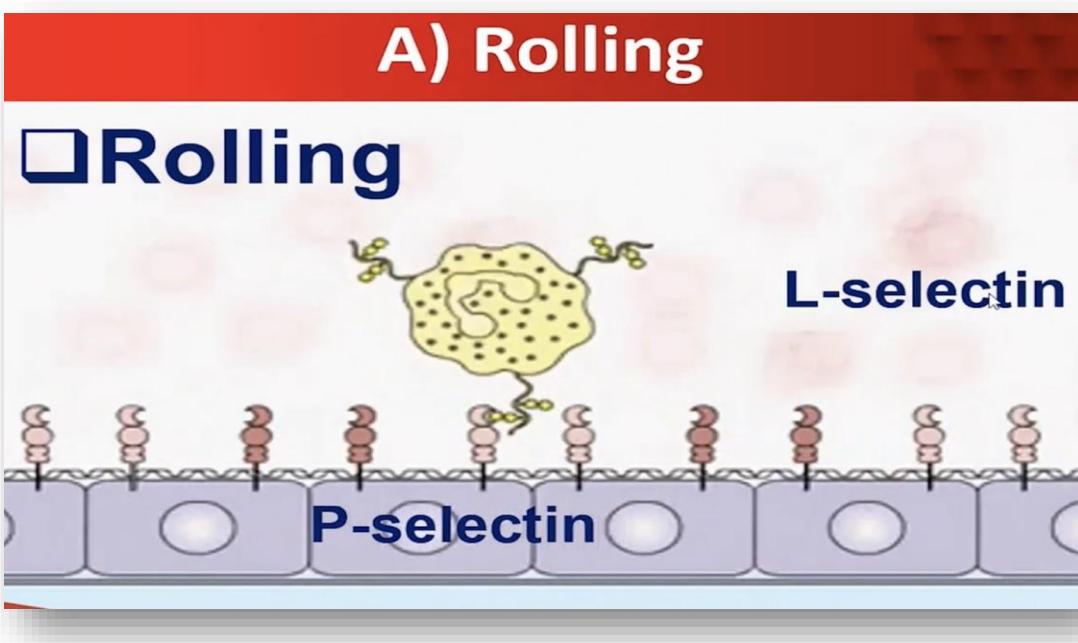
## A) Rolling



MO at the site of tissue injury release cytokines IL-1 & TNF- $\alpha$  which bind to their receptors on endothelial cells and lead to shrinking of endothelial cells and leakage of fluids and proteins into tissue (swelling)



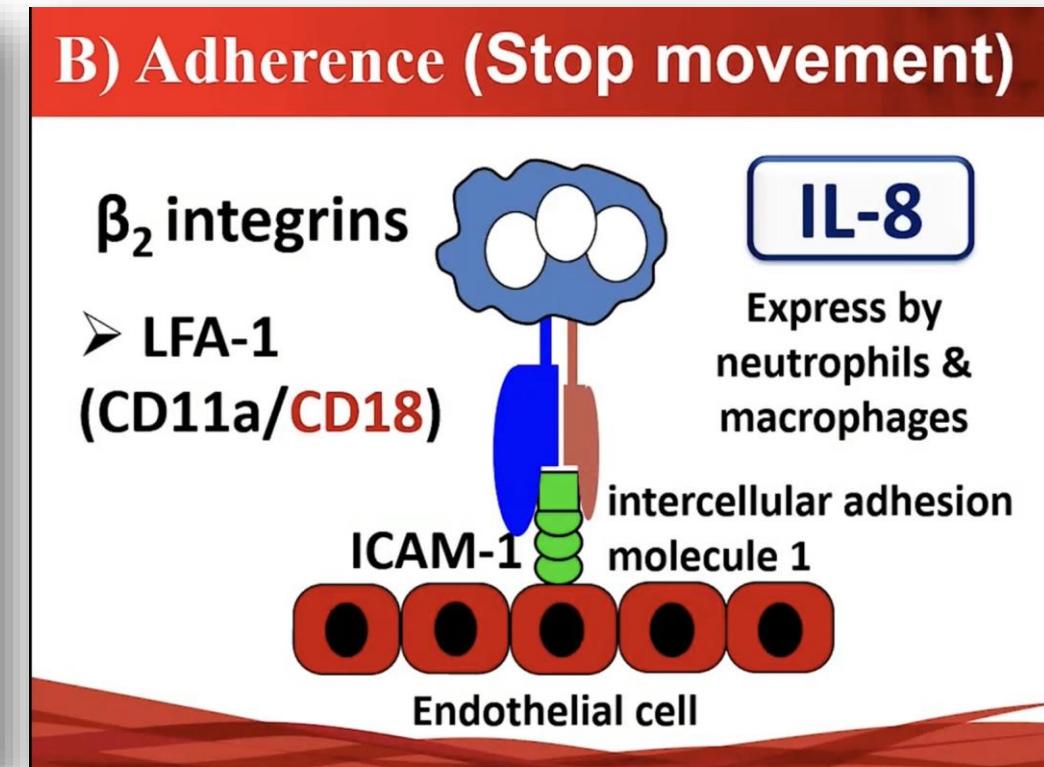
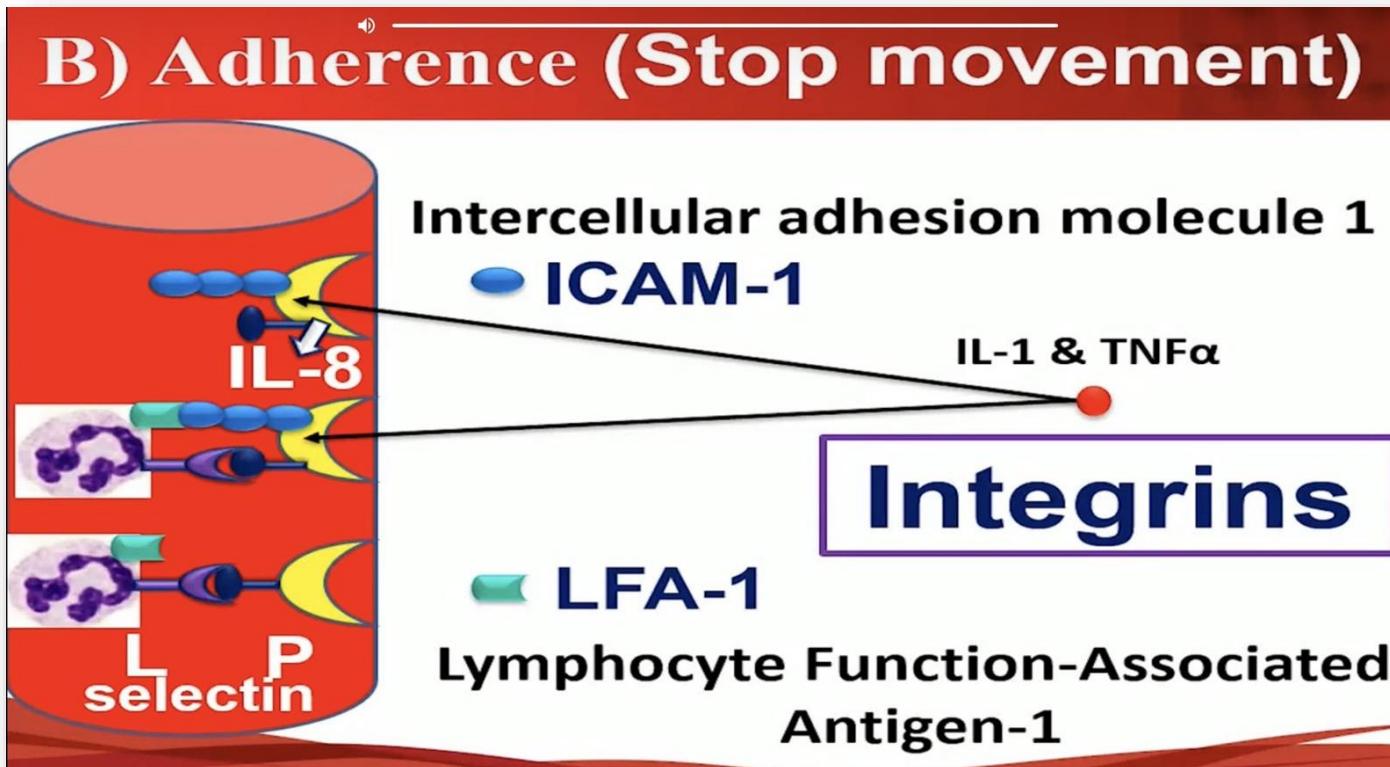
Cytokines IL-1 & TNF- $\alpha$  activate endothelial cells to express adhesion molecules E, P-selectin (sticky molecules). These cytokines enter the blood and induce WBCs to express L-selectin which leads to rolling of WBCs



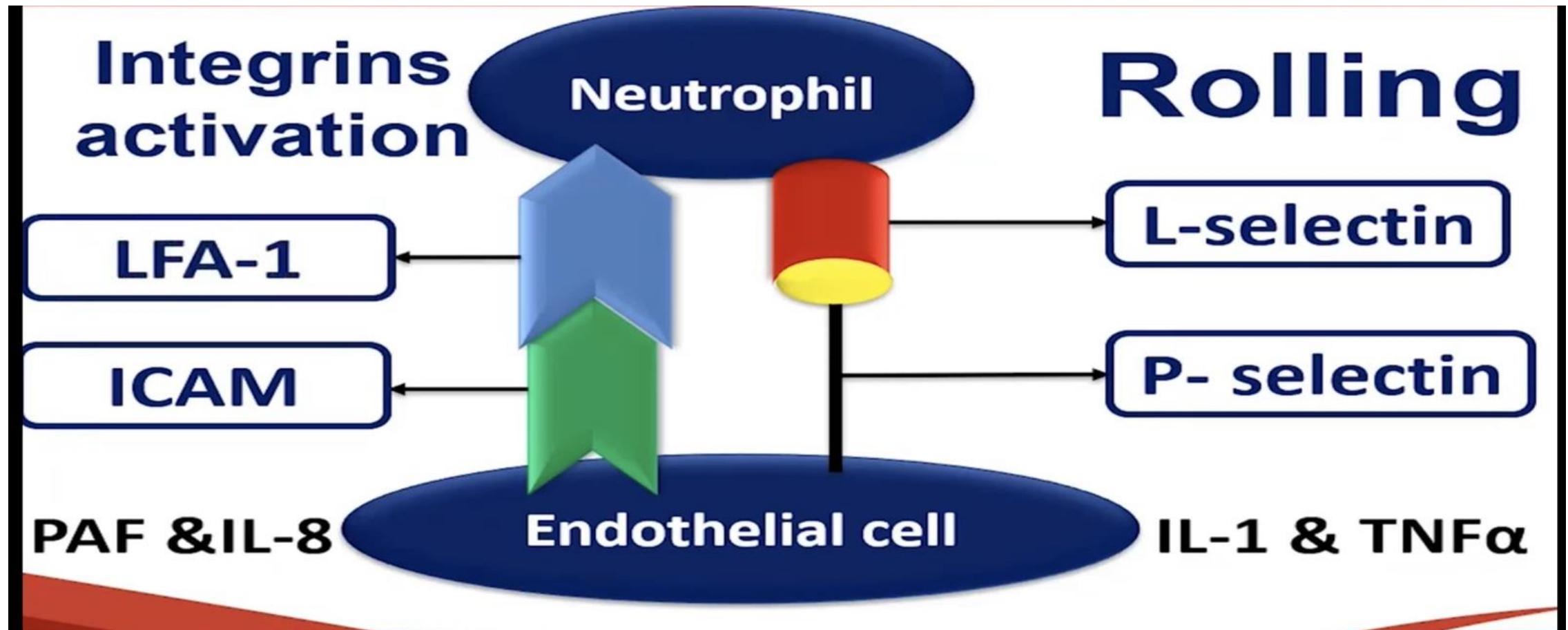
In rolling step just slow down the flow of WBCs so after that comes role of adherence or stop movement in which integrins play a role

## B- Adherence (Stop movement)

Cytokines IL-1 & TNF- $\alpha$  activate endothelial cells to produce IL-8 which in turn autoactive endothelial cells to produce a type of integrin called ICAM-1 and induce WBCs to express LFA-1



Both rolling and adherence require adhesion molecules which made by endothelial cells and leukocytes under the action of cytokines



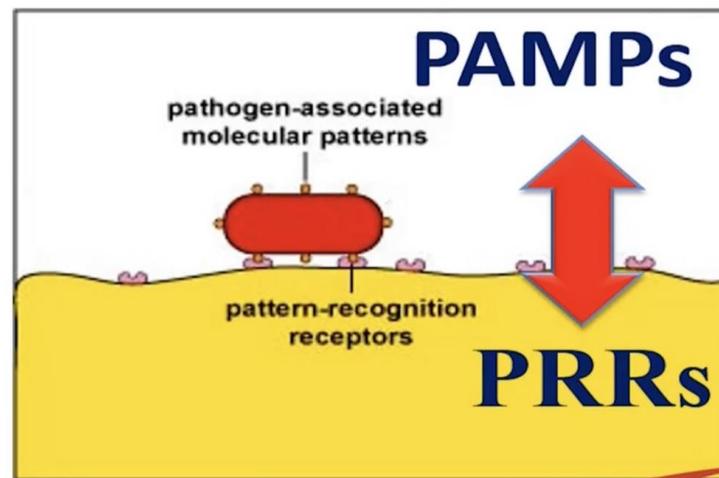
## What will happen in newborn with genetically deficiency of integrins?

- Swelling and redness at site of inflammation
- No WBCs at the site of inflammation
- Recurrent infection
- Leukocyte Adhesion Deficiency (LAD)

## 3- Attachment

### 3) Attachment

#### Attachment



### 3) Attachment

#### PAMPs

- Peptidoglycan
- Lipopolysaccharides
- lipoteichoic acids
- Flagellin
- ssRNA
- dsDNA

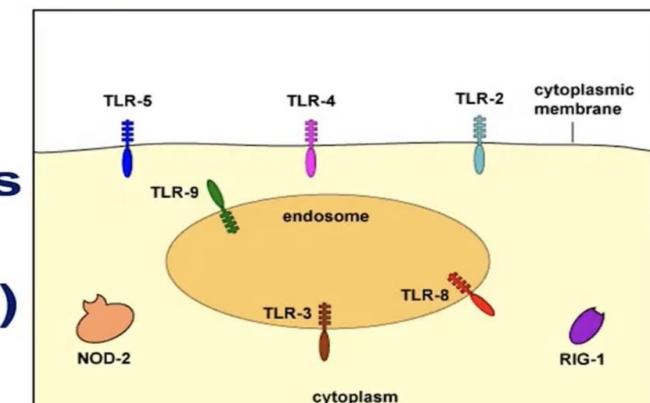
**Pathogen associated molecular patterns**

A red capsule-shaped organism is shown with several purple dots representing pathogen-associated molecular patterns (PAMPs) on its surface. The text 'Organism' is written inside the capsule.

### 3) Attachment

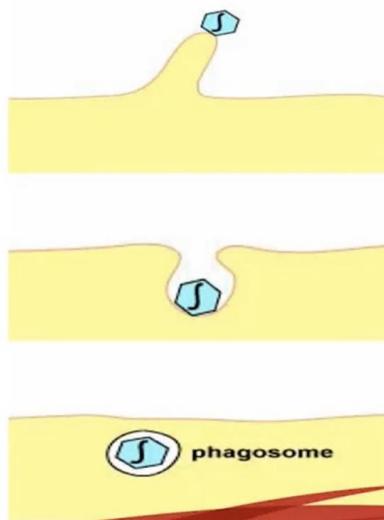
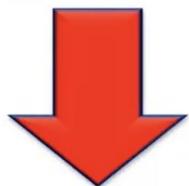
#### Pattern Recognition Receptors (PRR)

- Toll-like receptors (TLRs)  
(Phagocytic cells)



## 4) Ingestion

Engulfing by extending pseudopods around it

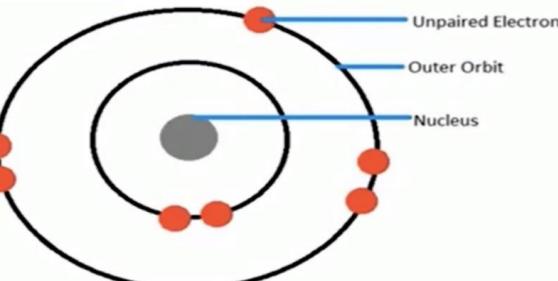


Phagosome formation

## 5) Killing By direct ( $O_2$ )

Free radicals

Unstable & highly reactive



Killing

Single unpaired electron in an outer orbit

## 5) Killing

Direct  
(Oxygen dependent)

$O_2$

Indirect  
(Oxygen independent)



## 5) Killing

Direct (Oxygen dependent)



NADPH Oxidase



Myeloperoxidase

Free radicals

## 5) Killing By direct (O<sub>2</sub>)

### Free radicals

#### Reactive oxygen species (ROS)

- Superoxide anion
- Hydrogen peroxide
- Hydroxyl radical

(Respiratory burst)

Killing

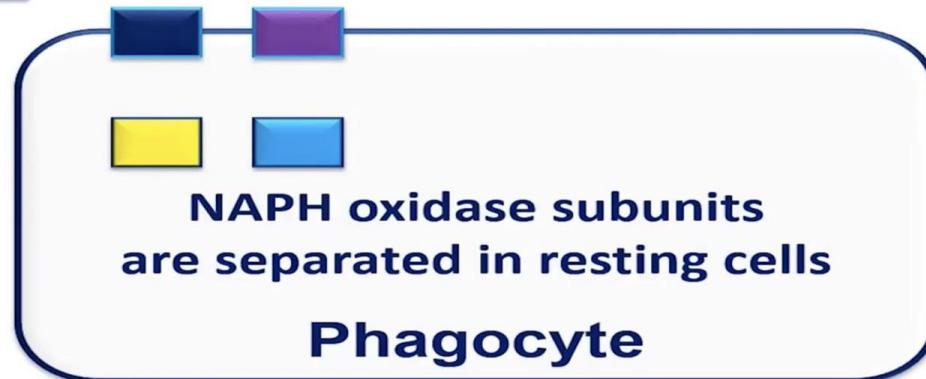
#### Reactive nitrogen species (RNS)

- (Nitric oxide)

## NADPH Oxidase complex



Resting macrophage

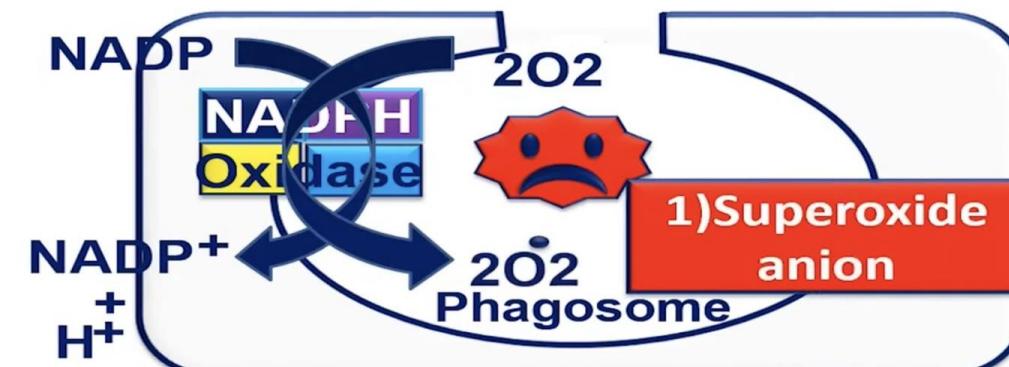


Phagocyte

## H<sub>2</sub>O<sub>2</sub> formation



## Superoxide inside phagosome



## Hydroxyl radical formation

3) Hydroxyl radical



## Hypochlorous formation

Myeloperoxidase (MPO)

4) Hypochlorous



Bleach

## Reactive nitrogen species (RNS)

IFN- $\gamma$  (autocrine)

5) Nitric oxide

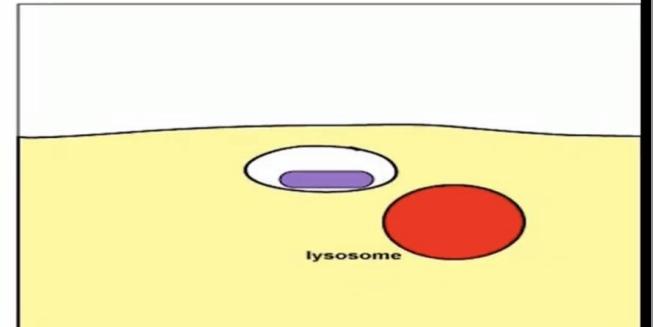


inducible nitric oxide synthase (iNOS).

## 5) Killing

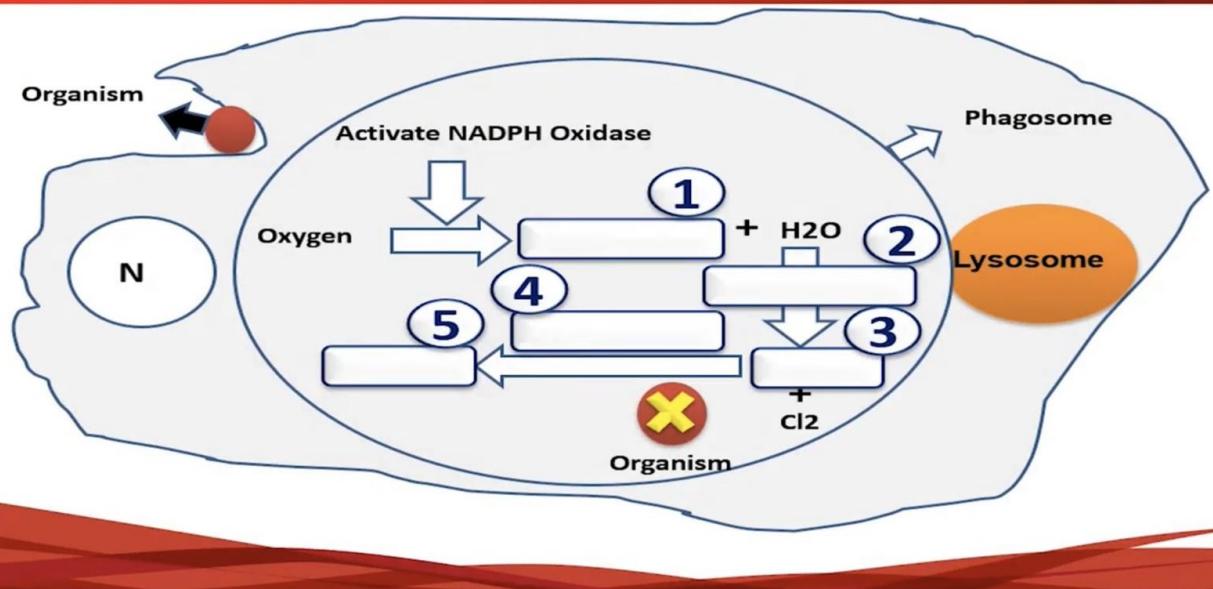
### Indirect (Oxygen independent)

- Hydrolytic & Proteolytic enzymes

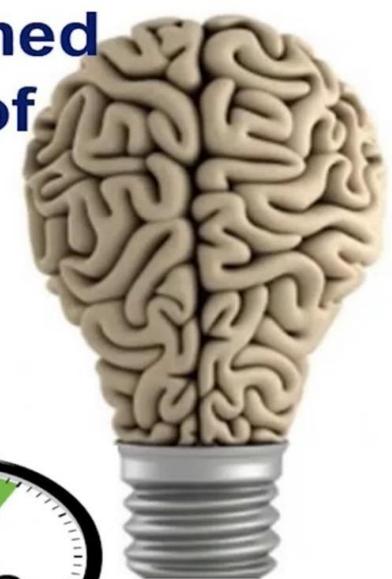
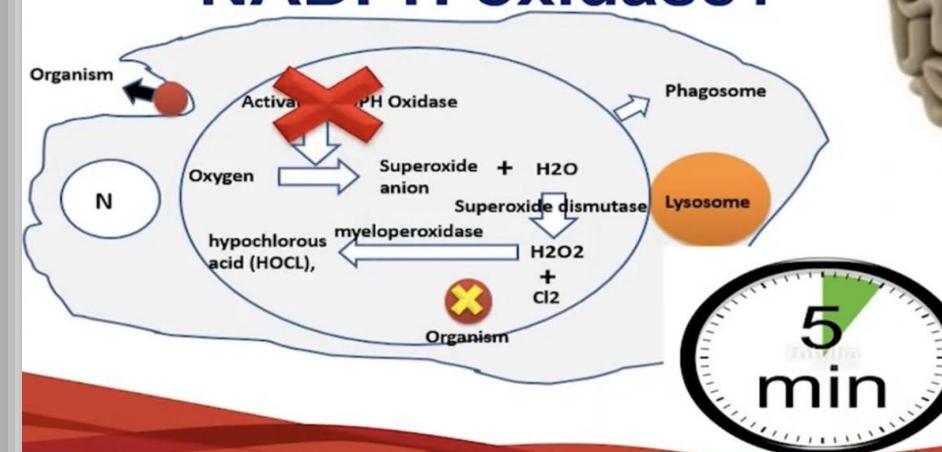


It is worth to know that hydrolytic enzymes become activated when there is free radical formation within phagosomes

### Exercise 3



Predict, What is happened  
If there is an absence of  
NADPH oxidase?



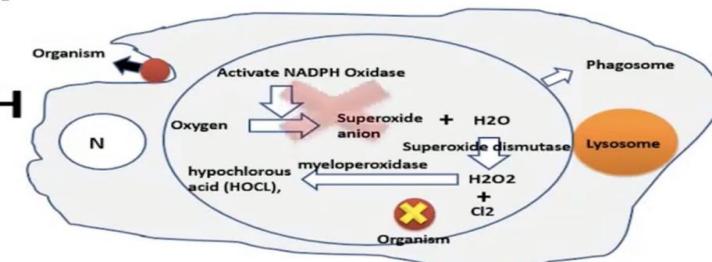
In CGD patient suffers from recurrent bacterial infection especially *Staph.aureus*, while not with *Strep.,pneumoniae* infection .

Children with G6PD also suffering from recurrent bacterial infection because don't have NADPH enzymes

Chronic granulomatous disease (CGD)

Deficiency of NADPH oxidase

(Recurrent infection)



## Chediak Higashi Syndrome

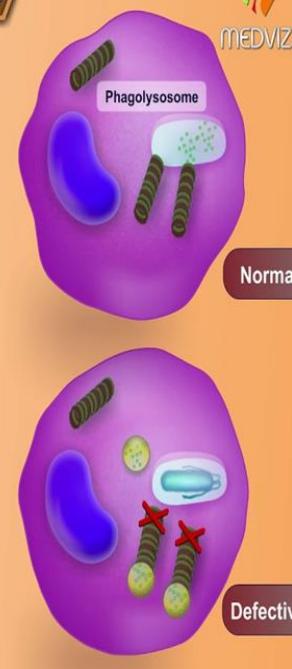
MEDVIZZ

### Pathophysiology

Defect in microtubule function prevents fusion of lysosome with phagosome

→ Bactericidal defect

↓  
Increased susceptibility to staphylococcus aureus infections

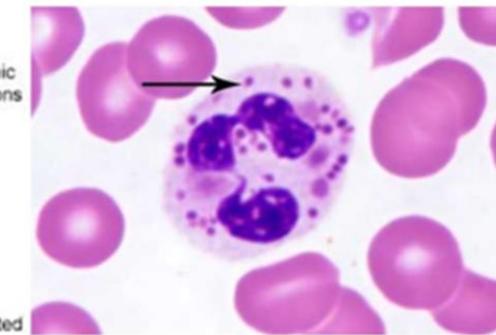
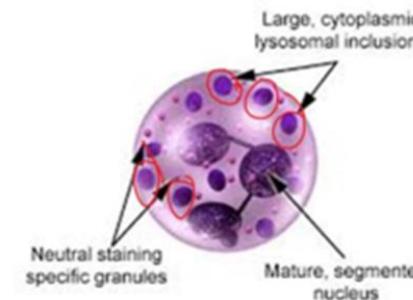


## Chédiak-Higashi syndrome

- A form of **phagocyte bactericidal dysfunction** characterized by unusual oculo-cutaneous albinism, high incidence of **lymphoreticular neoplasms**, and recurrent **pyogenic infections**
- A defect in **microtubule function** prevents **lysosomes** from fusing with **phagosomes** to produce a **phagolysosome**

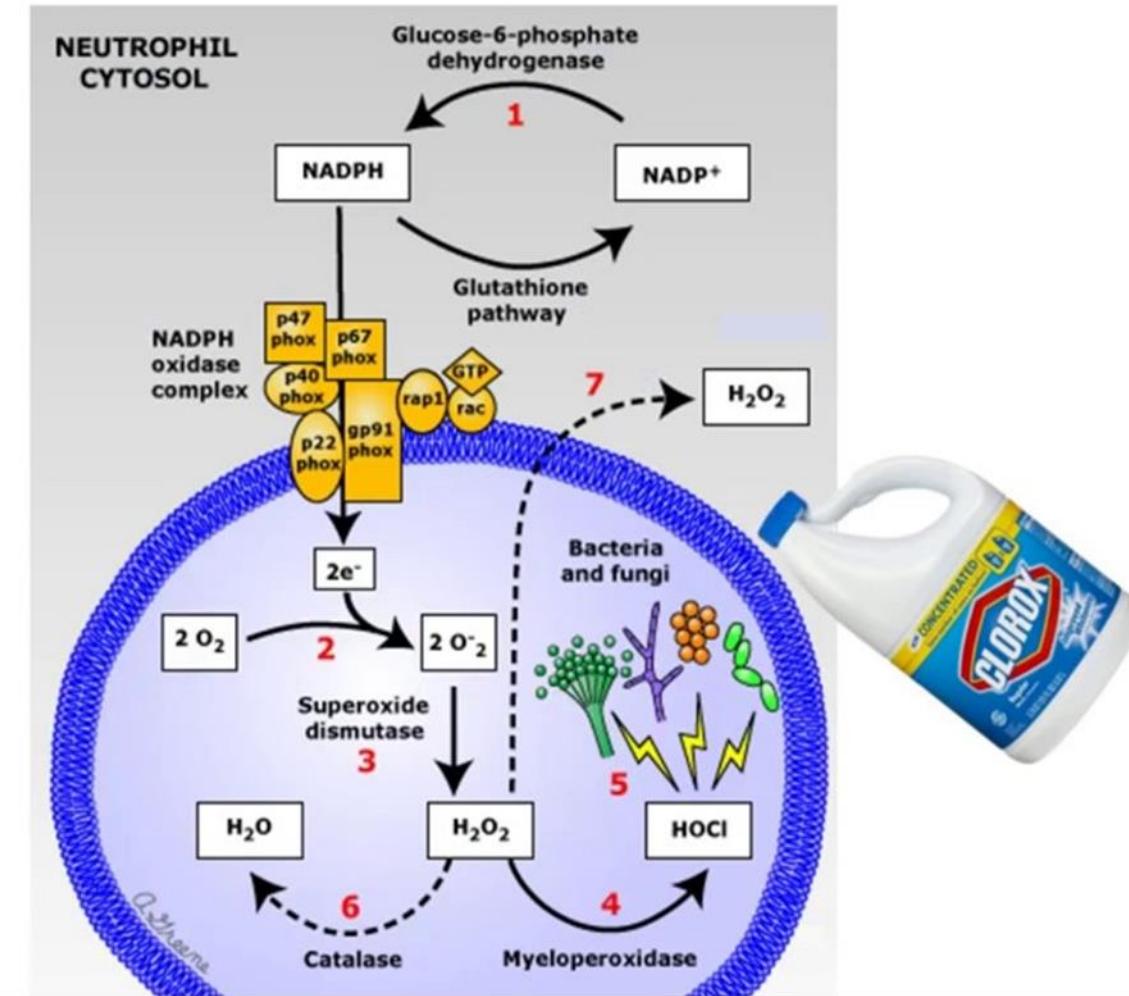


CHEDIAK-HIGASHI NEUTROPHIL



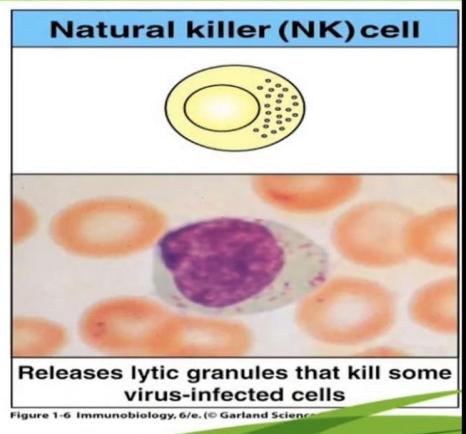
What happens if there is genetically deficiency in NADPH enzyme structure?

# Why Does CGD Make You Sick?



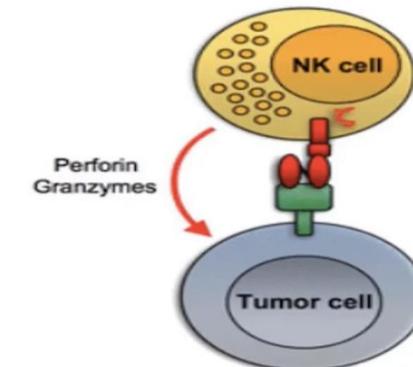
### III) Cytotoxicity (NK cells)

- Large granular lymphocytes
- CD16 & CD56



### III) Cytotoxicity (NK cells)

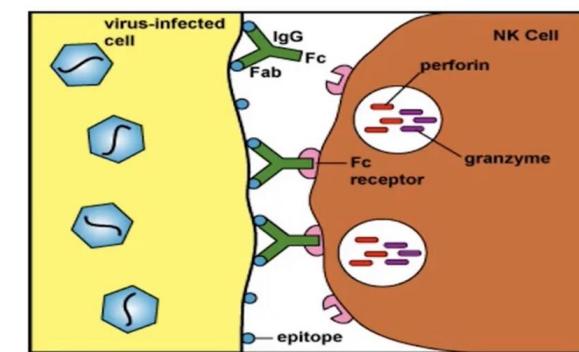
- Direct killing
- Killing virus infected cells
- Tumor cell



### III) Cytotoxicity (NK cells)

Indirect killing

**Antibody Dependent Cellular Cytotoxicity (ADCC)**



The highly mobile cells that are the first to arrive at the site of an infection, are the

Monocytes

Neutrophils

Macrophages

Eosinophils

Why is neutrophil the first cell come out in tissue acute inflammation

Most motile

Abundant number

Chemotactic factors

Eosinophil

Rolling phase occurs through

P selectin with E selectin

P selectin with L selectin

LFA-1 with L selectin

LFA-1 with ICAM1

Pathogen-associated molecular patterns (PAMPs) recognize

Bacteria

T Cells

pattern recognition receptors

Toll like receptors

The main enzyme responsible for production of free radicals that destroy organism is

NADPH oxidase

Hydrogen peroxidase

Myeloperoxidase

Superoxide dismutase

Which of the following are reactive oxygen intermediate (ROI)?

Nitric oxide

Superoxide anion

Hydrogen peroxide

Hydroxyl radical

NK cells have the following characters:

Kill viral infected cells

Phagocytic cells

Part of innate Immunity

Kill tumor cells

Adherence process (stop movement) in migration of leukocyte occurs through

P selectin with E selectin

P selectin with L selectin

LAF-1 with L selectin

LAF-1 with ICAM1