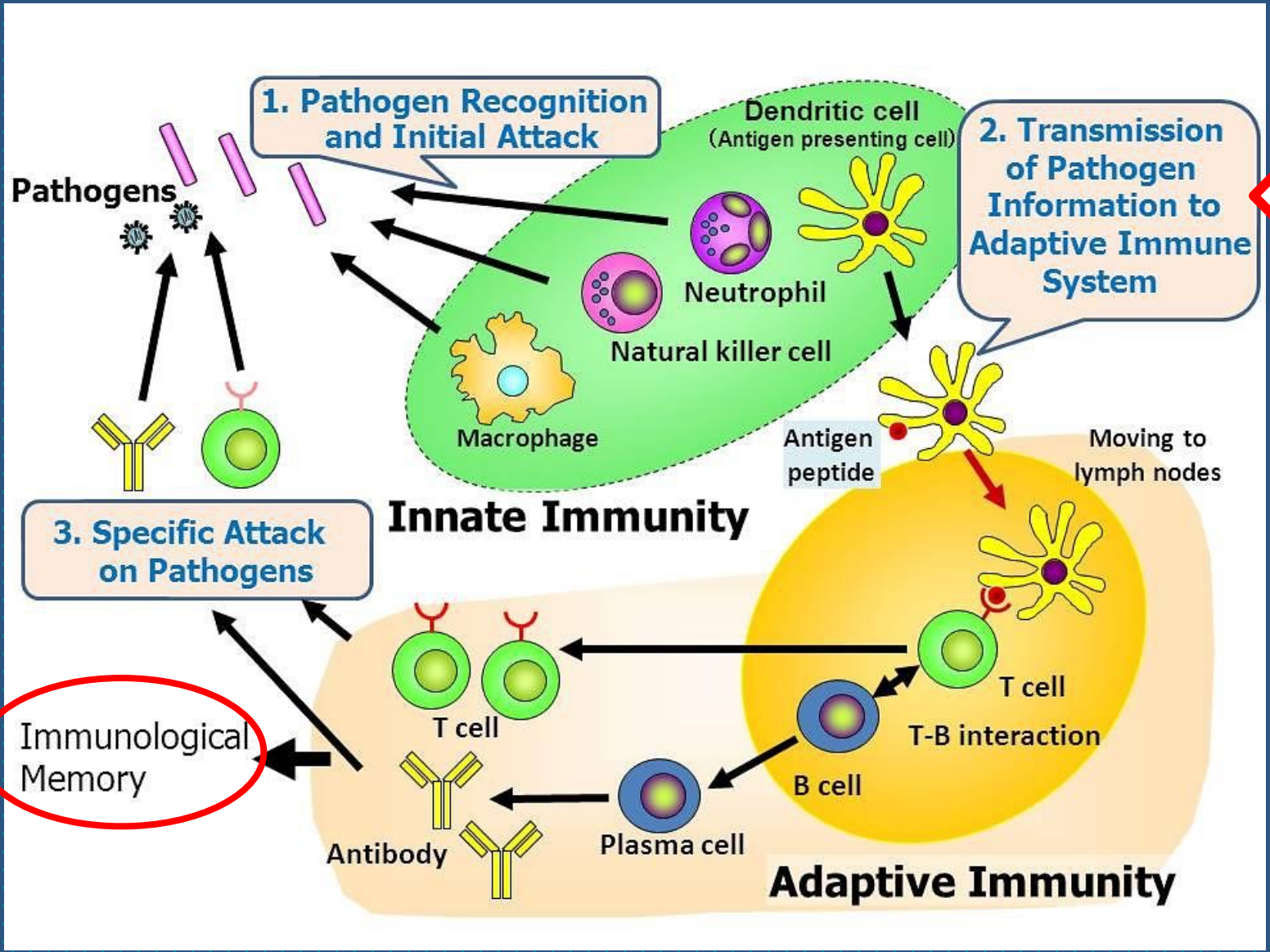
# Innate and adaptive immunity cohoperate to defeat infections



# Main functions of the immune system

- Recognizes, **destroys** and **clears** a variety of pathogens
- Recognizes and **clears damaged self components**
- Initiates **tissue and wound healing** processes

# Immunopathology

- As a branch of Immunology, **immunopathology** concerns disorders caused by alterations of the immune response
- 3 main categories of alterations:
  - excessive response ⟹ hypersensitivity reactions
  - inappropriate response ⟹ autoimmune diseases
  - defective response ⟹ immunodeficiencies

# Immunodeficiencies

- Primary (congenital)
- Secondary (acquired)

# Primary immunodeficiencies

- Congenital defects caused by mutations of genes coding for proteins involved in the regulation of innate and acquired immunity

- Such defects pertain to both humoral and cellular major components of the immune response

# Primary immunodeficiencies

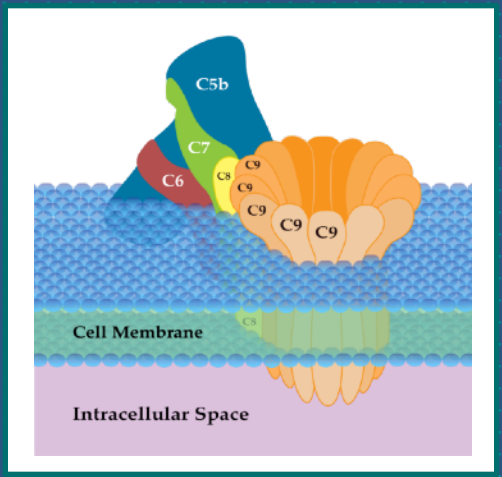
- Most diseases caused by primary immunodeficiencies arise **early during infancy** (4th-6th month to 2nd year of life)

- In general, patients affected by primary immunodeficiencies show high suscettibility to **recurrent**, life-threatening and often fatal **infections**

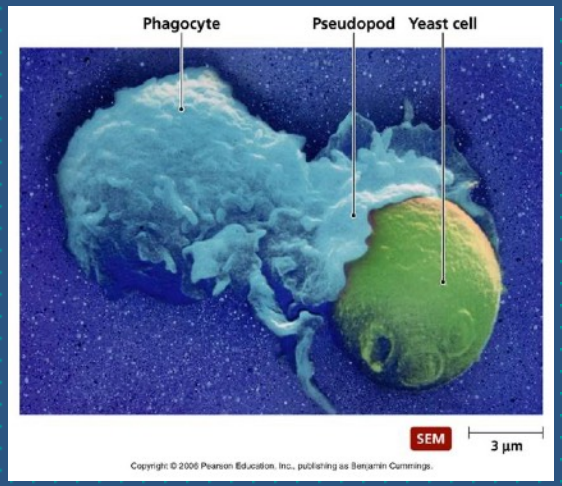
# Defects of **innate** immune response

**PRIMARY**
(congenital)
DEFECTS OF
**INNATE IR**

→ **HUMORAL** COMPONENT → COMPLEMENT SYSTEM




**CELLULAR** COMPONENT → PHAGOCYTES

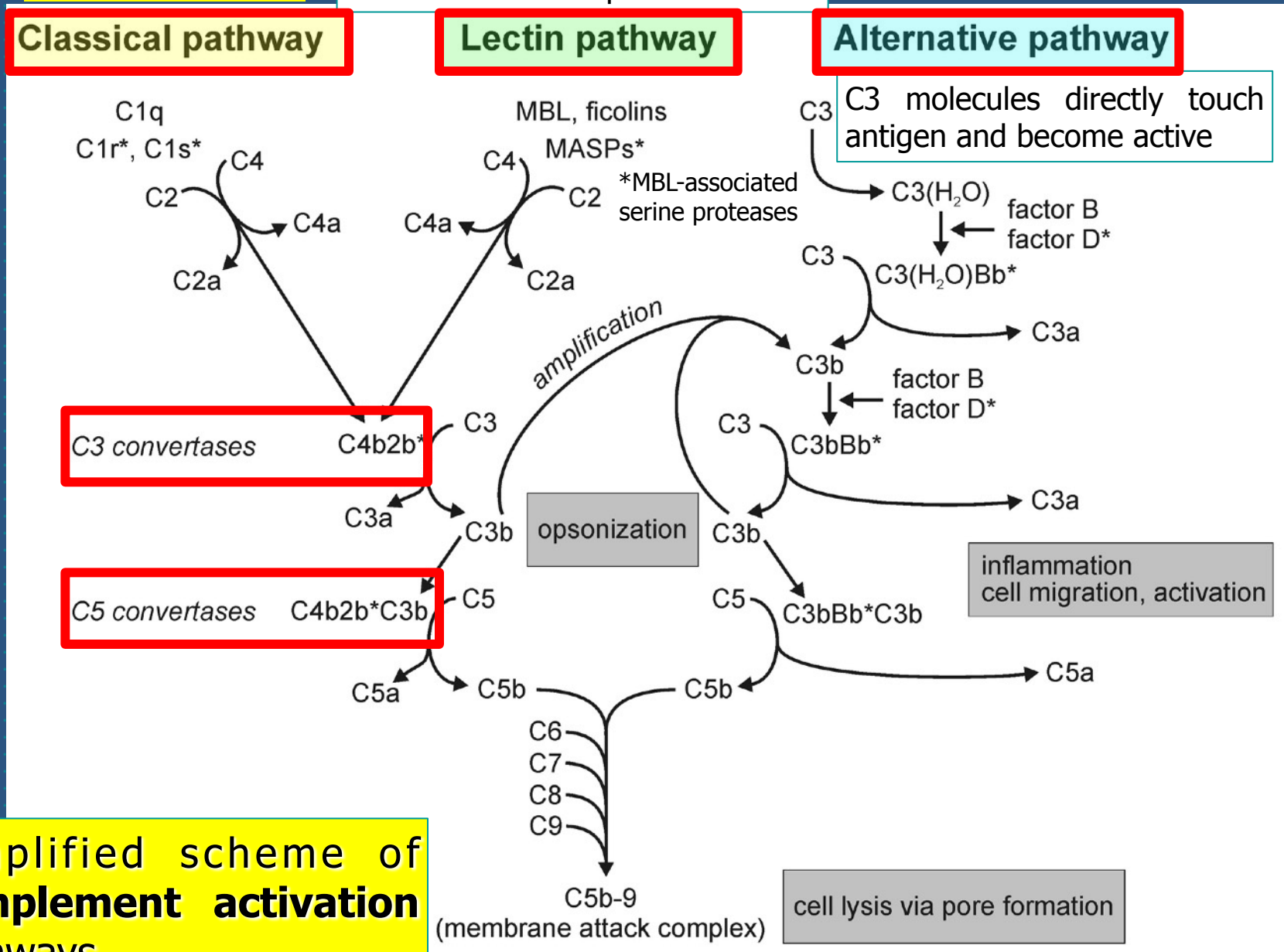


# The complement system

- The complement system consists of several plasma proteins (nearly 30, including regulatory elements) that work together to:
  - opsonize microbes
  - promote the recruitment of phagocytes to the site of infection (chemotaxis)
  - directly kill the microbes
- The first step in activation of the complement system is recognition of molecules on microbial surfaces but **not** host cells
- This occurs in **three ways**, each referred to as a distinct pathway of complement activation

**Ab-dependent**

The **lectin** recognizes and binds carbohydrates of the target cell which activate complement




Simplified scheme of **complement activation** pathways

# Roles of the major complement components (1)

- Recognition of microbes by any of the three complement pathways results in sequential recruitment and assembly of additional complement proteins into **complexes** with **protease activity**
- One of these complexes, called **C3 convertases**, cleaves the central protein of the complement system, C3, producing C3a and C3b
- The larger **C3b fragment** serves as an opsonin to promote phagocytosis of the microbes
- The smaller **C3a fragment** is released and stimulates inflammation by acting as a chemoattractant for neutrophils and an enhancer of blood vessels permeability

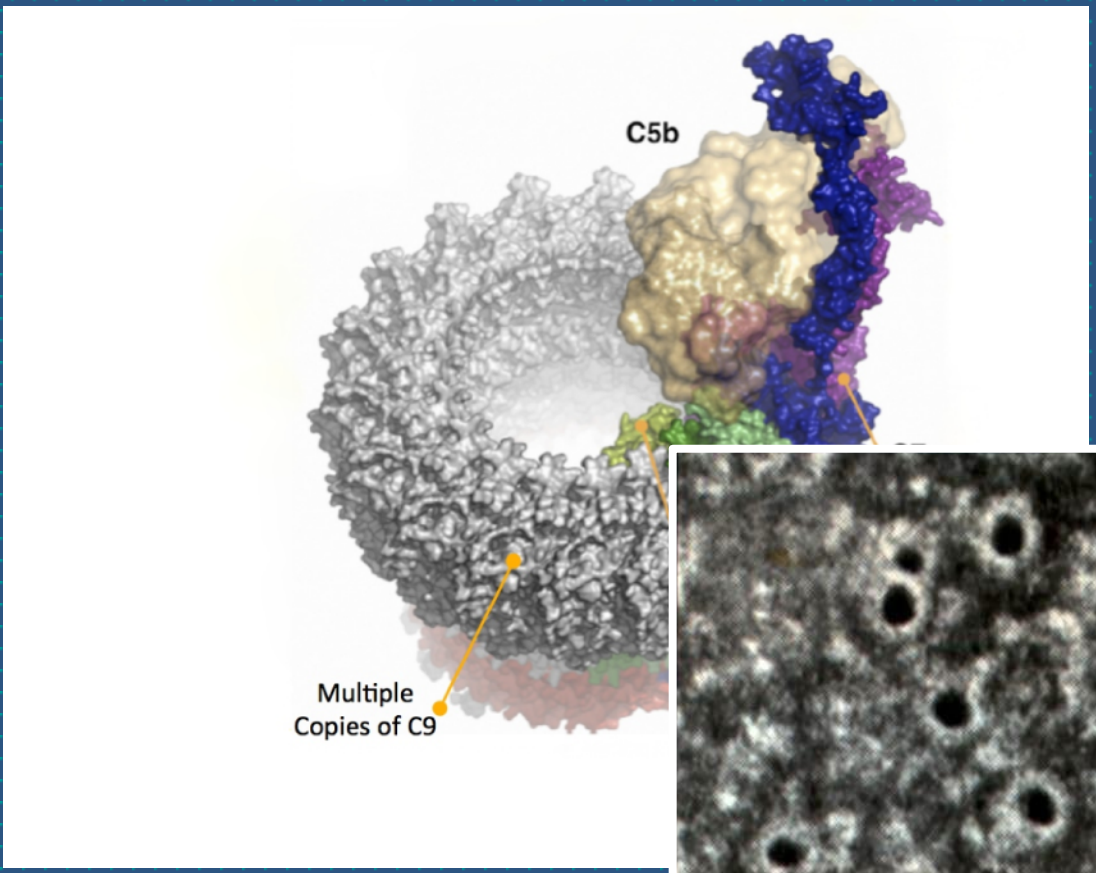
# Roles of the major complement components (2)

- **C3b** binds other complement proteins to form a protease called **C5 convertase** that cleaves C5, generating a released peptide (C5a) and a larger fragment (C5b) that remains attached to the microbial cell membranes

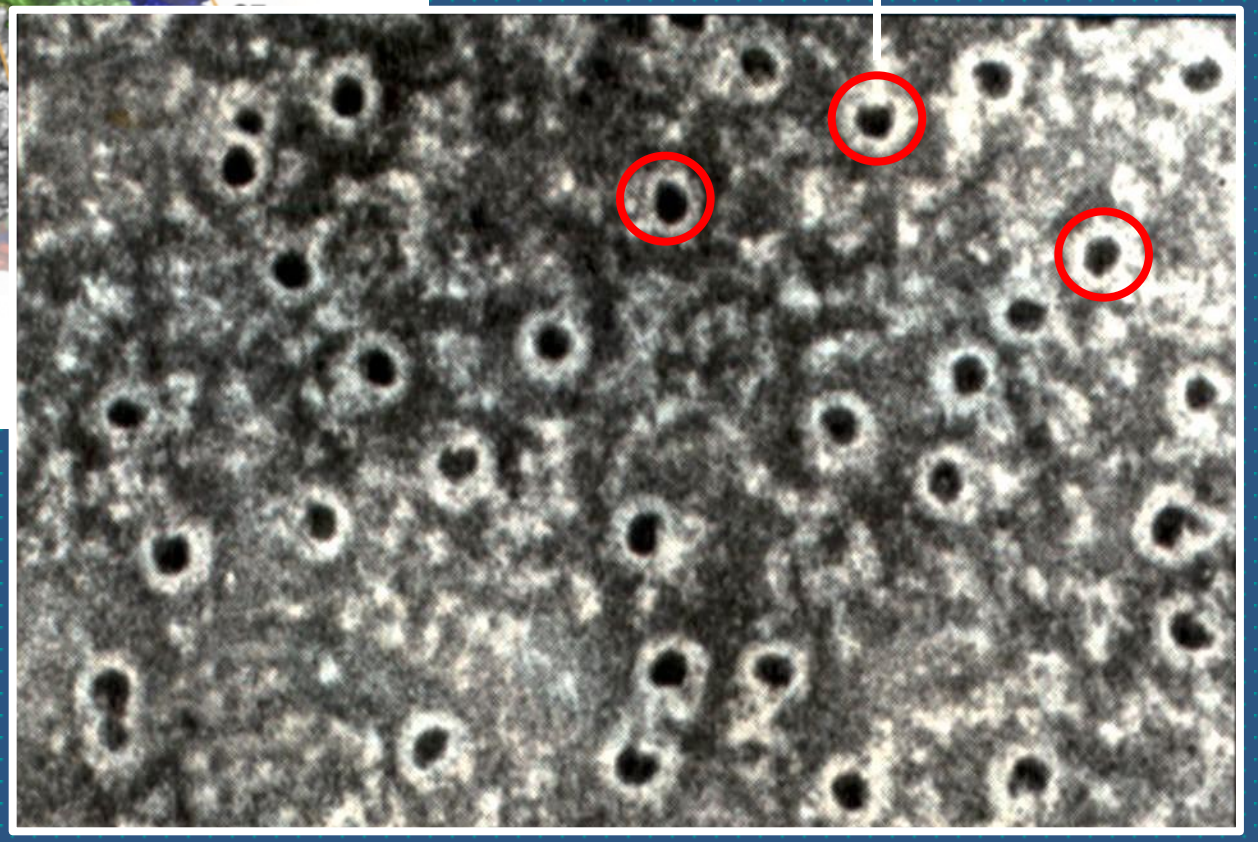
- **C5a** is also a chemoattractant; in addition, it enhances blood vessels permeability that make them leak plasma proteins and fluid into sites of infections (C5a >>> C3a)

- **C5b** initiates the formation of a complex of the complement proteins C6, C7, C8, and C9, which are assembled into a membrane pore called the membrane attack complex (MAC) that causes lysis of the cells where complement is activated

# MAC assembly into a target cell membrane




MAC hole on a target cell



# Major complement system deficiencies

- The complement system is an essential component of innate immunity
- Patients with deficiencies in complement components are mostly susceptible to recurrent, often lethal, **bacterial infections**

| Deficient complement protein | Effects of deficiency |
|---|---|
| C3 | Profound susceptibility to frequent serious pyogenic infections (fatal in early life) |
| C2 (the most common), C4 | Increase incidence of immune complex diseases (akin to Systemic Lupus Erythematosus syndrome); failure to clear circulating immune complexes by the classic pathway |
| C5, C6, C7, C8, C9 | Disseminated infections by *Neisseria* spp |

# Innate immune response to Neisseria spp infection

- The genus Neisseria contains two important human pathogens, N. gonorrhoeae and N. meningitidis.
- N. gonorrhoeae (gonococcus) infections have a high prevalence and low mortality, whereas N. meningitidis (meningococcus) infections have a low prevalence and high mortality
- Both gonococcus and meningococcus can escape killing by neutrophils and therefore **complement** activation is **crucial** to defeat infection
- Individuals with inherited complement deficiencies have a markedly increased risk of acquiring neisserial infections

# N. gonorrhoeae

- **Gonorrhea** is a specific type of **urethritis** that involves mucous membranes of the urethra, resulting in a copious discharge of pus, more apparent in the male than in the female
- Survival of gonococci inside the phagocytes is accounted for by gene products which protect against oxidative and non-oxidative components made by neutrophils
- Gonococcus-derived catalase degrades hydrogen peroxide (produced during neutrophil respiratory burst)
- Gonococcus pili (hair-like appendages) and por protein reduce neutrophil granule fusion with the membrane of phagosomes

# N. meningiditis

- Meningitis refers to inflammation of the meninges of the brain or spinal cord
- N. meningitidis has a prominent antiphagocytic polysaccharide capsule
- Meningococcal capsule contributes to protect the bacteria against cationic antimicrobial peptides
- N. meningiditis capsule contains sialic acid, a compound known to inhibit complement activation

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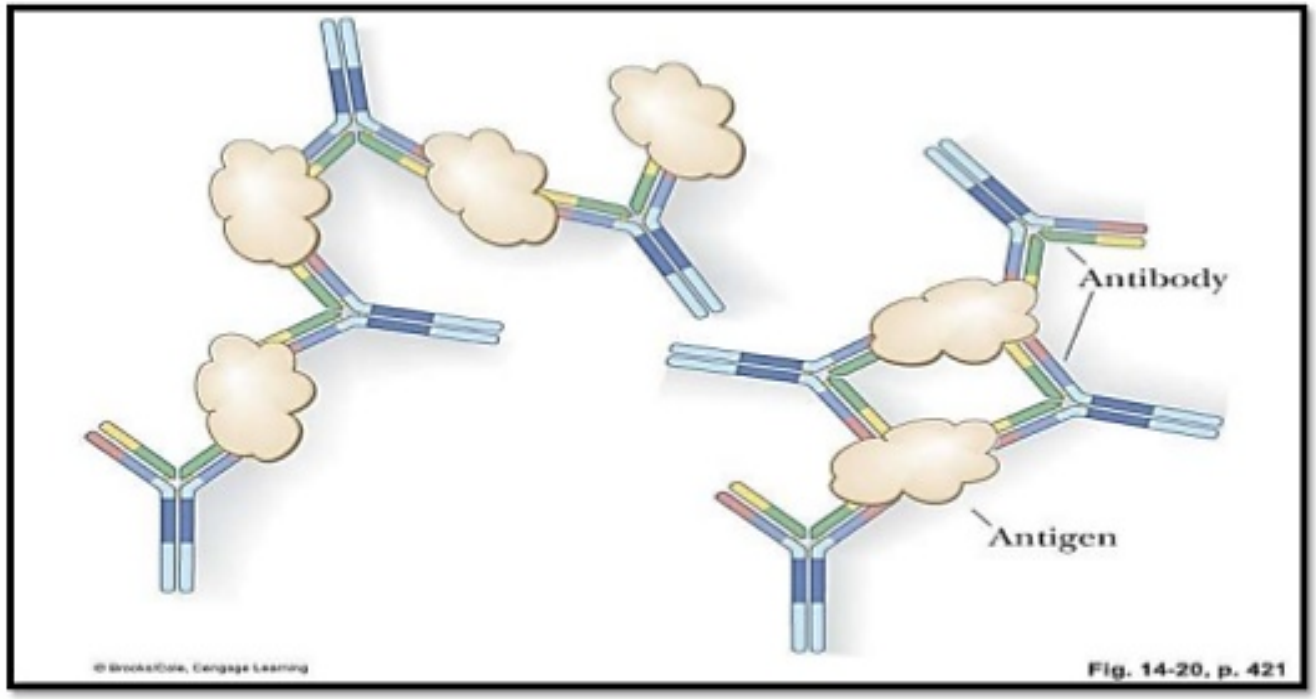
# Complement-mediated clearance of immune complexes

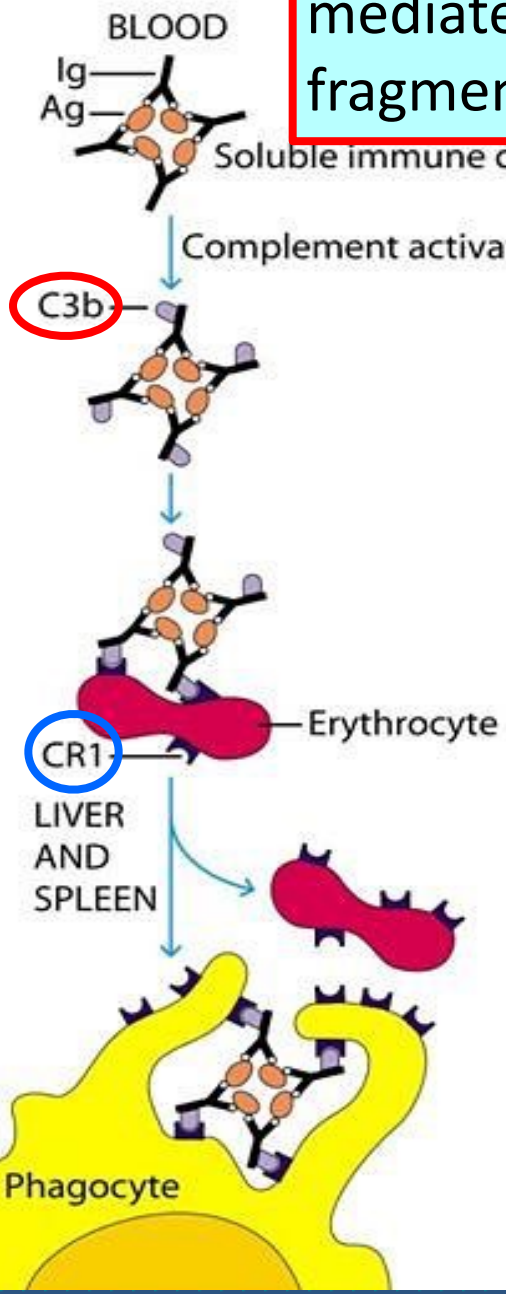
## 2. IMMUNE COMPLEX:

An immune complex is formed from the integral binding of an antibody to a soluble antigen.

Ag + Ab → Ag-Ab complex



**Clearance of circulating immune complexes** is mediated by receptors (**CR1**) for complement **C3b** fragment on erythrocyte plasmamembrane




**C3b** formation and binding to complexes

- Erythrocyte-bound immune complexes are transported by the blood stream to liver and spleen
- Macrophages residing in these tissues have **more CR1** than erythrocytes: they strip and ingest the complexes carried by the passing red blood cells
- Deficiencies of C1, C2 and C4 (**crucial for C3 convertase** assembly and **C3b formation**) are associated with an increased predisposition for developing **immune complexes diseases** (e. g. SLE)

**Primary** immune deficiencies caused by alterations of **immune cell** functions

# Professional phagocytes

- **Neutrophils, monocytes** and **macrophages** are key players in fighting invading microorganisms
- They engulf, kill and destroy microbes, mostly bacteria and fungi
- Their **crucial role** in such a protective response is witnessed by **rare maladies**, almost all hereditary in nature, in which **absence or alteration of molecules** that preside phagocyte functions are unavoidably **associated with severe infections**

# Congenital immunodeficiencies caused by defects in innate immunity

| Disease | Functional Deficiencies | Mechanisms of Defect |
|---|---|---|
| Chronic granulomatous disease | Defective production of reactive oxygen species by phagocytes | Mutations in genes encoding components of the phagocyte oxidase enzyme, most often cytochrome b558 |
| Leukocyte adhesion deficiency-1 | Absent or deficient expression of β2 integrins causing defective leukocyte adhesion–dependent functions | Mutations in gene encoding the β chain (CD18) of β2 integrins |
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| Chédiak-Higashi syndrome | Defective lysosomal function in neutrophils, macrophages, and dendritic cells, and defective granule function in natural killer cells | Mutation in a gene encoding a lysosomal trafficking regulatory protein |

REVIEW

CURRENT OPINION

# Lessons from rare maladies: leukocyte adhesion deficiency syndromes

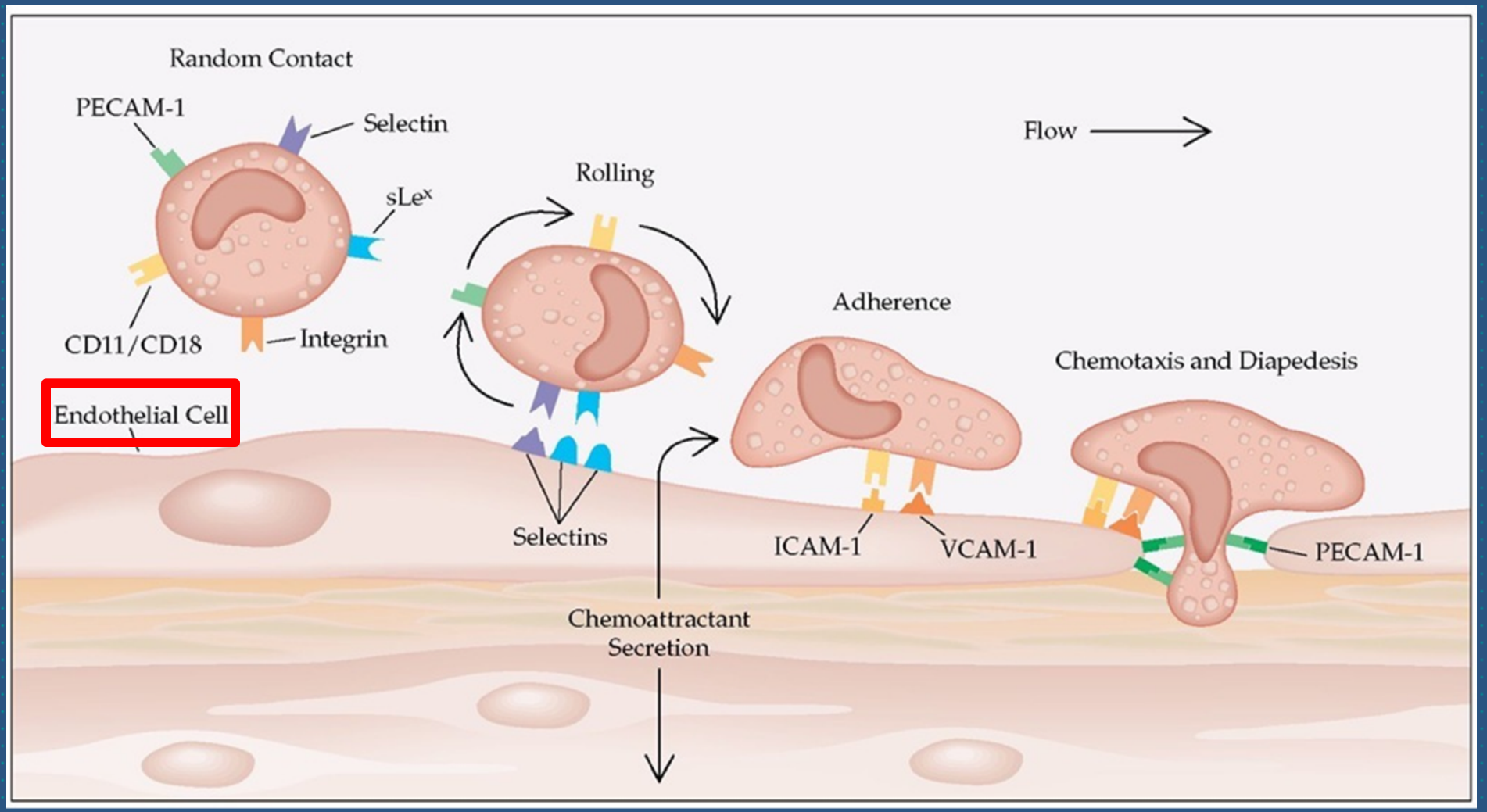
*Estelle S. Harris*[a], *Andrew S. Weyrich*[a,b], *and Guy A. Zimmerman*[a]

Leukocyte adhesion deficiency (LAD) syndromes: rare (≤1:1,000,000 live births) primary immunodeficiencies classified as defects in adhesion-dependent functions of phagocytes, mainly **neutrophils** and **monocytes**

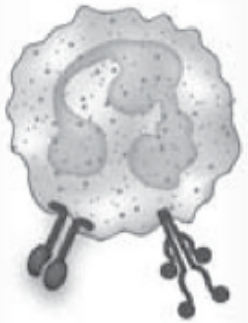
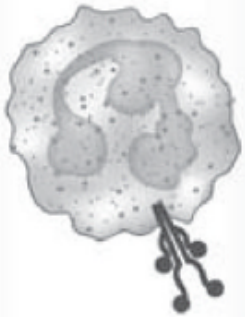
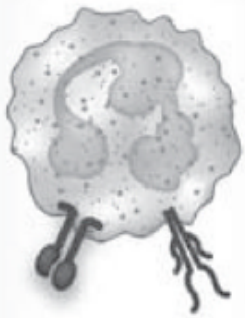
Two main syndromes:

- Leukocyte adhesion deficiency – type 1 (**LAD-1**)
- Leukocyte adhesion deficiency – type 2 (**LAD-2**)

# The neutrophil migration cascade during inflammation: from random contact to diapedesis

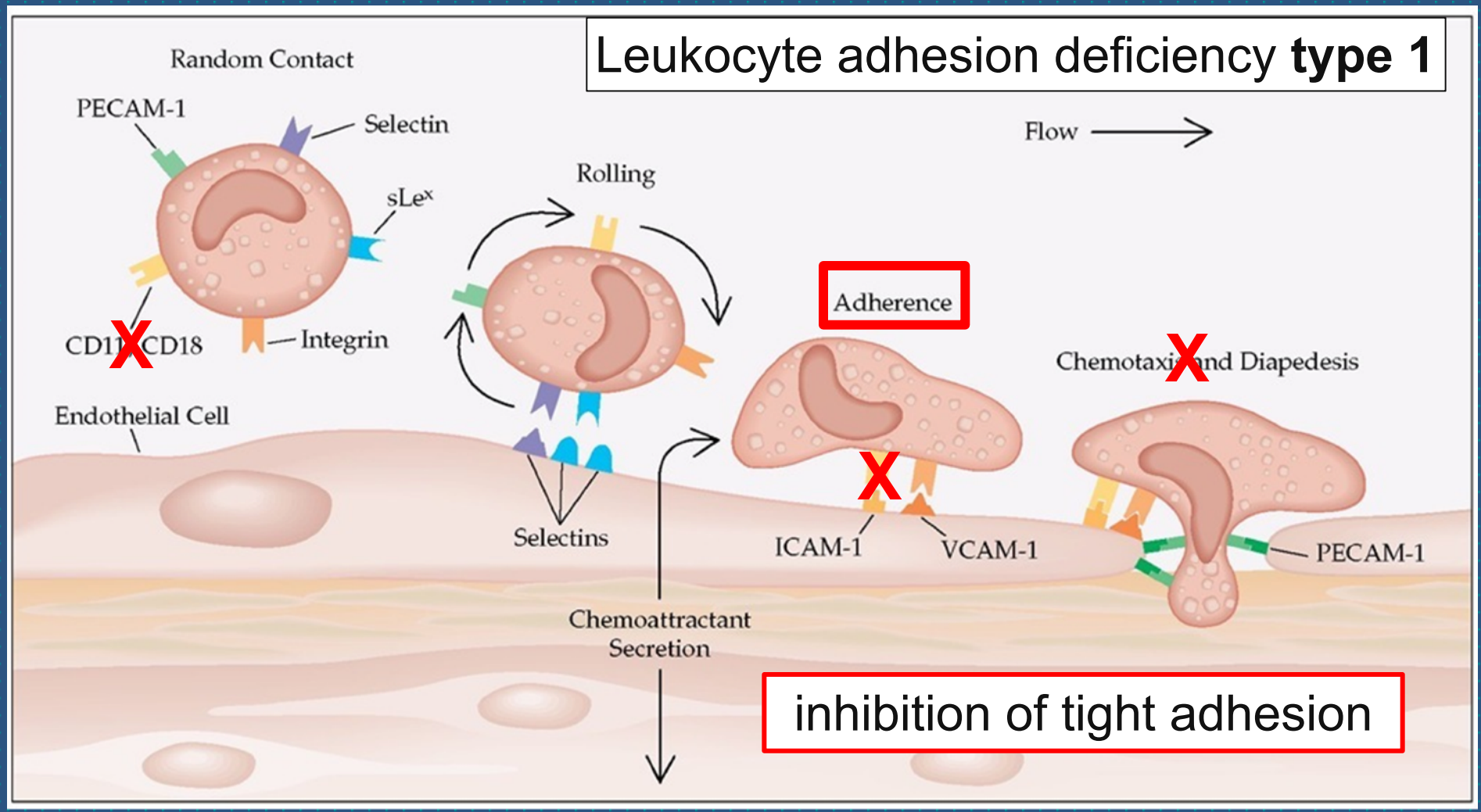


# Leukocyte adhesion deficiencies

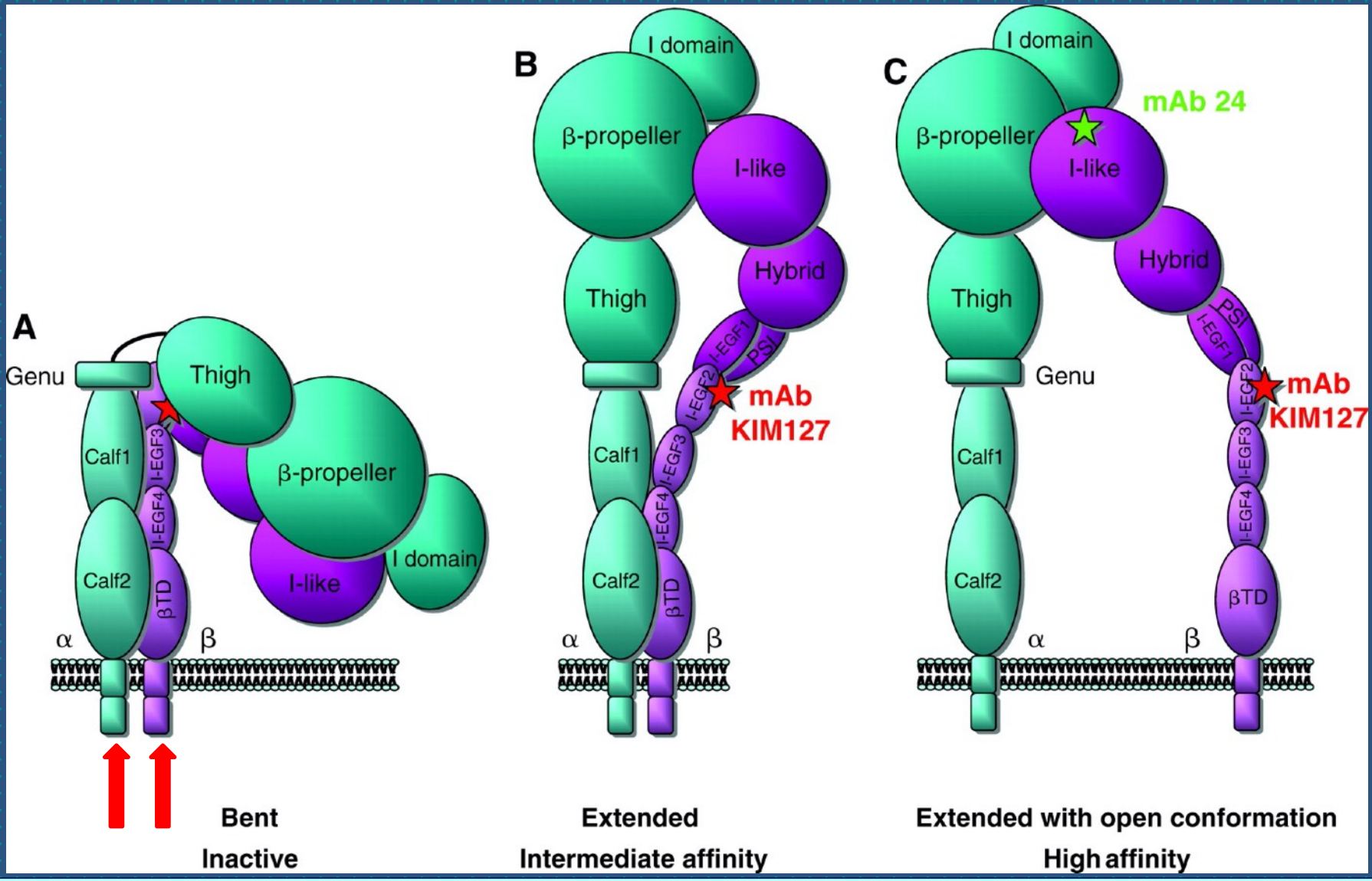
| | Normal | LAD-I | LAD-II |
|---|---|---|---|
| |  |  |  |
| Integrins | Present | Absent or decreased $\beta_2$ integrins | Normal |
| Selectin ligands | Present | Normal | Defective fucosylation |
| Functional defects | None | Tight adhesion, emigration | Rolling |
| Mutations | None | *ITGB2* | *SLC35C1* |

# Defects in neutrophil migration

Defective **firm adhesion** to endothelial cell surface impairs neutrophil diapedesis and migration to infected tissue



# β2 integrin heterodimers

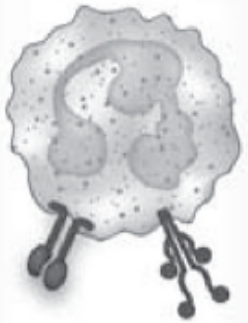
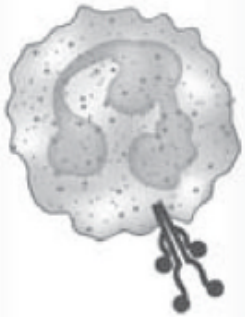
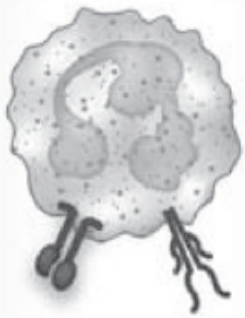



Signals induced by many common neutrophil stimuli **enhance** $\beta_2$ integrin **affinity** by acting on their **cytoplasmic domains**

# LAD-1: absence or reduced expression of beta-2 integrins on neutrophils

- Inflammatory lesions lack neutrophil infiltrate
- Patient's neutrophils can roll but **do not stick** on endothelial cells
- **Neutrophilia**: a lot of neutrophils in the circulation
- Recurrent bacterial infections, delayed wound healing, delayed detachment of residual umbilical cord
- In severe cases of LAD I (< 1% expression of $\beta_2$ chain), patients succumb to life-threatening infections, usually within 2 years of life

# Leukocyte adhesion deficiencies

| | Normal | LAD-I | LAD-II |
|---|---|---|---|
| |  |  |  |
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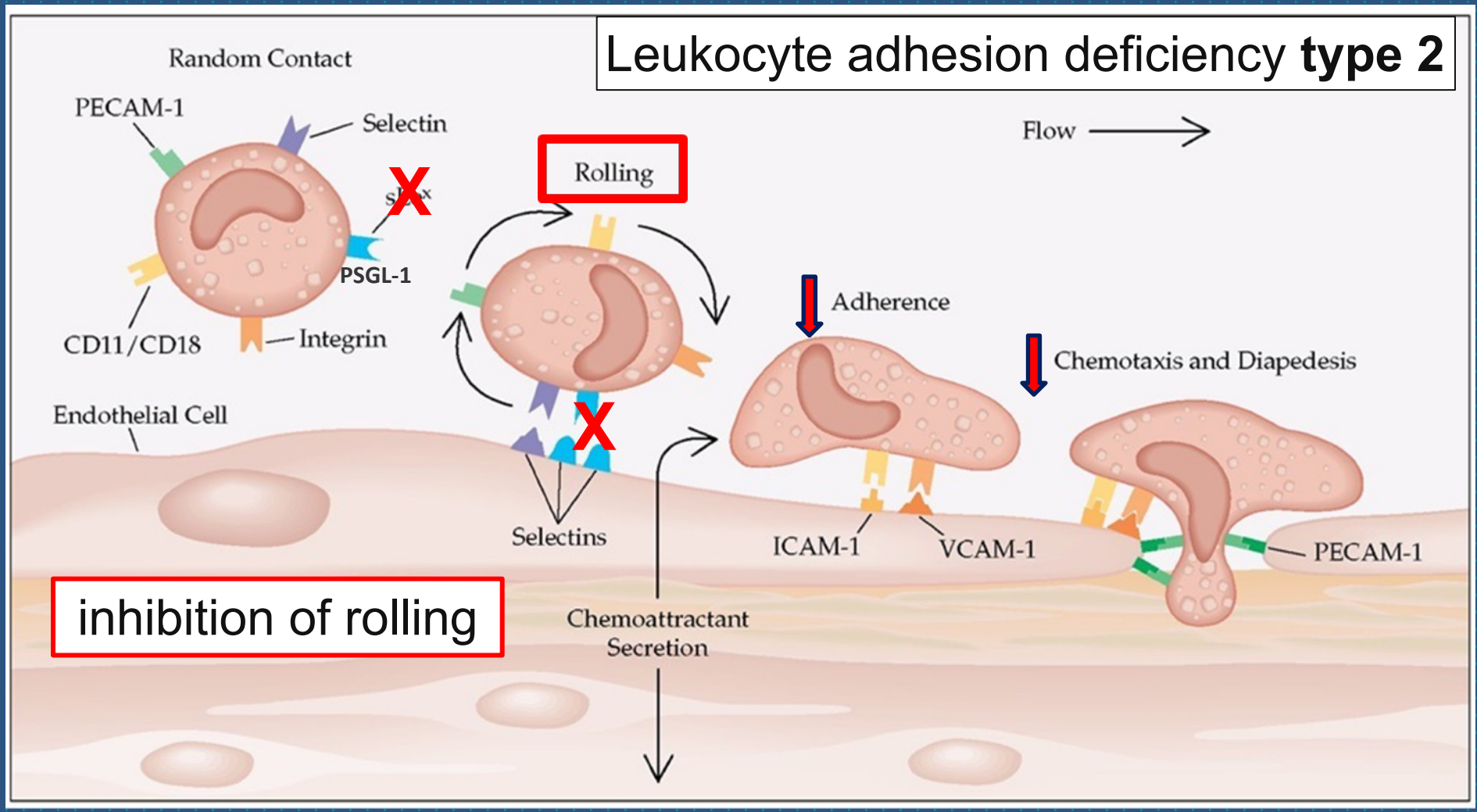
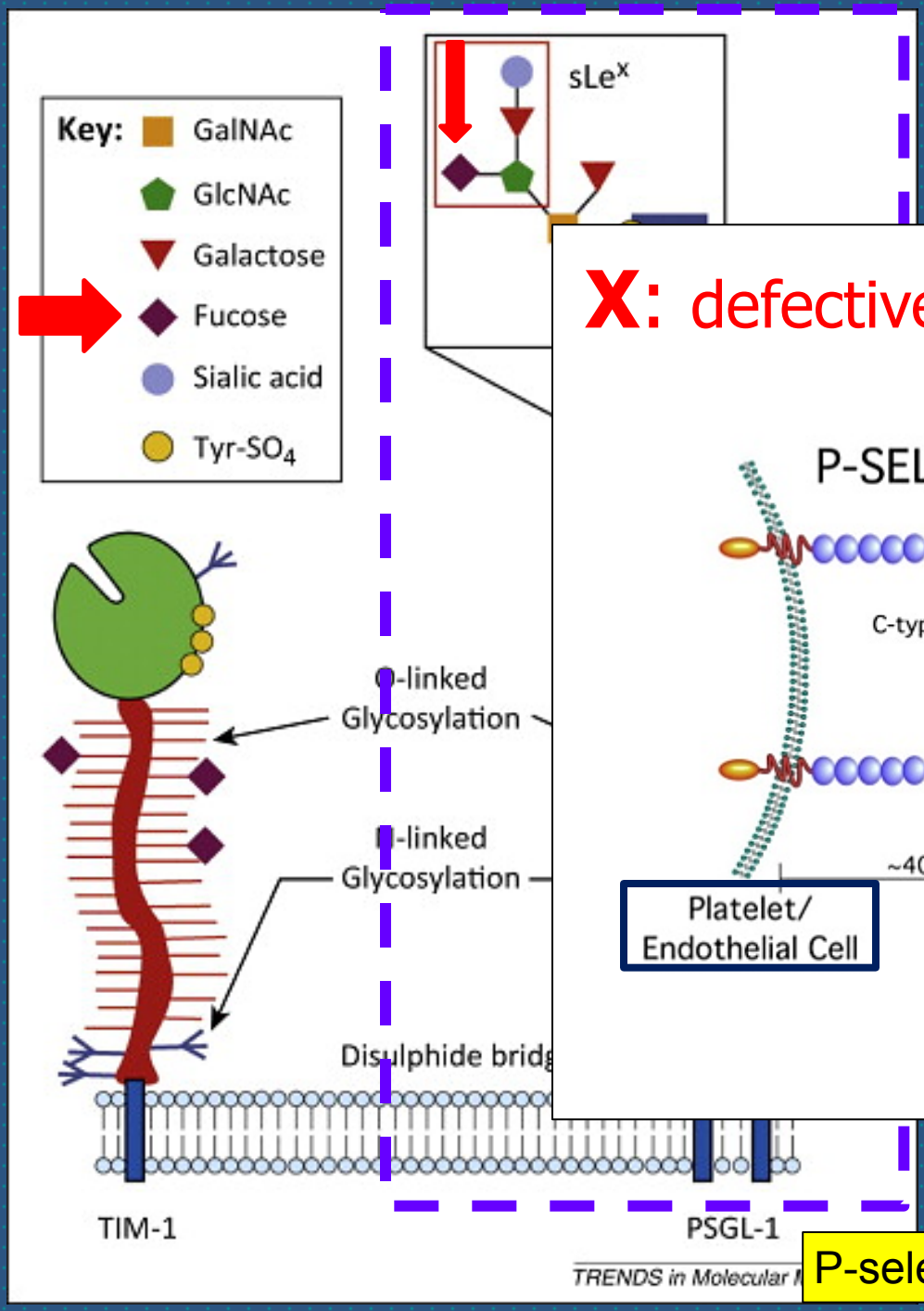
Defective rolling on endothelial cell surface impairs neutrophil diapedesis and migration to infected tissue



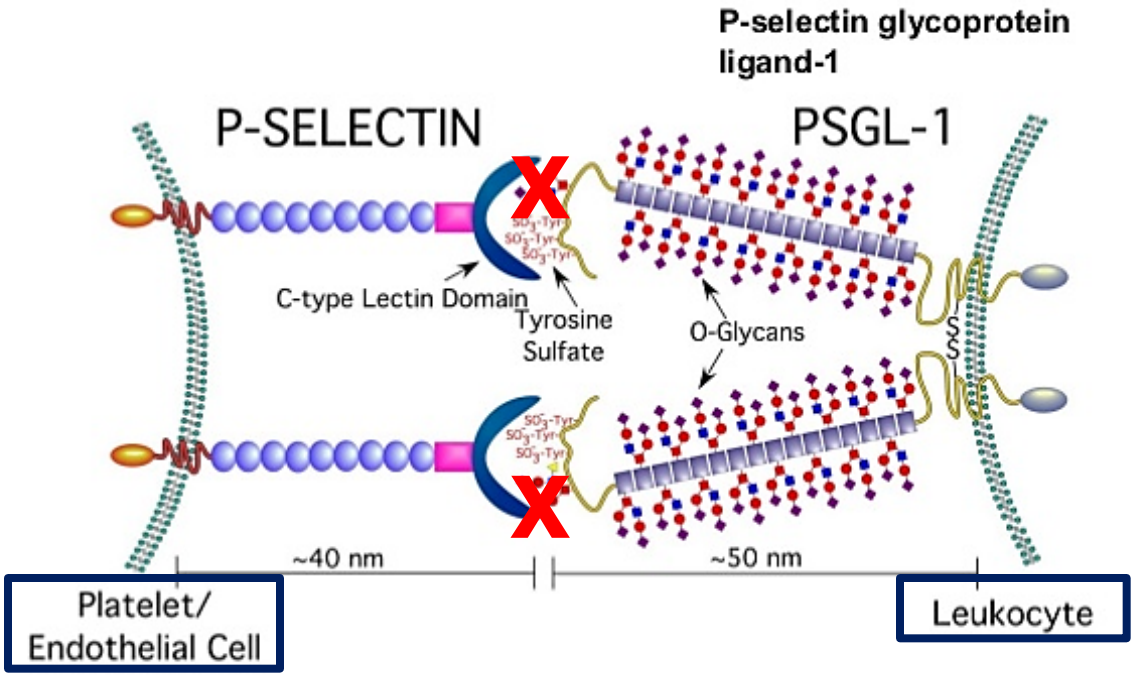
# LAD-2

- LAD -2 is caused by mutations in the gene encoding for a fucose specific transporter present in the Golgi membrane
- Impaired fucosylation of **sialyl Lewis X** on P-selectin glycoprotein ligand 1 (PSGL-1, expressed on neutrophils) that is the ligand for endothelial selectins (E- and P-selectins) reduces neutrophil-endothelial cell interaction

# Sialyl Lewis X




**X:** defective in LAD-2




P-selectin glycoprotein ligand 1

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- Neutrophilia
- Recurrent infections
- Delayed wound healing

# Clinical features of identified LAD-2 patients

**Table I** Clinical Features of Patients with LAD II

| | Israeli Arab boy [9, 10] | Israeli Arab boy [9, 10] | Israeli Arab boy [4] | Israeli Arab girl [4] | Turkish boy [12] | Brazilian boy [14] | Pakistani girl [14] |
|---|---|---|---|---|---|---|---|
| Recurrent infections | + | + | + | + | + | + | + |
| Neutrophilia | + | + | + | + | + | + | + |
| Mental retardation | + | + | + | + | + | + | + |
| Short stature | + | + | + | + | + | + | + |
| Coarse face | + | + | + | | | | + |
| Autistic features | + | + | | | + | | + |
| Convulsions | + | + | + | | + | + | + |
| Cerebral atrophy | + | + | + | + | + | | |
| Microcephaly | + | + | | | + | + | + |
| Intrauterine growth retardation | + | | | | + | | |

Alterations in metabolic pathways causing the severe **psychomotor** and **growth retardation** are still unclear

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ASBMT
American Society for Blood and Marrow Transplantation

# Chronic Granulomatous Disease: Lessons from a Rare Disorder

*Brahm H. Segal,[1] Paul Veys,[2] Harry Malech,[3] Morton J. Cowan[4]*

*Biol Blood Marrow Transplant 17: S123-S131 (2011)* © *2011 American Society for Blood and Marrow Transplantation*

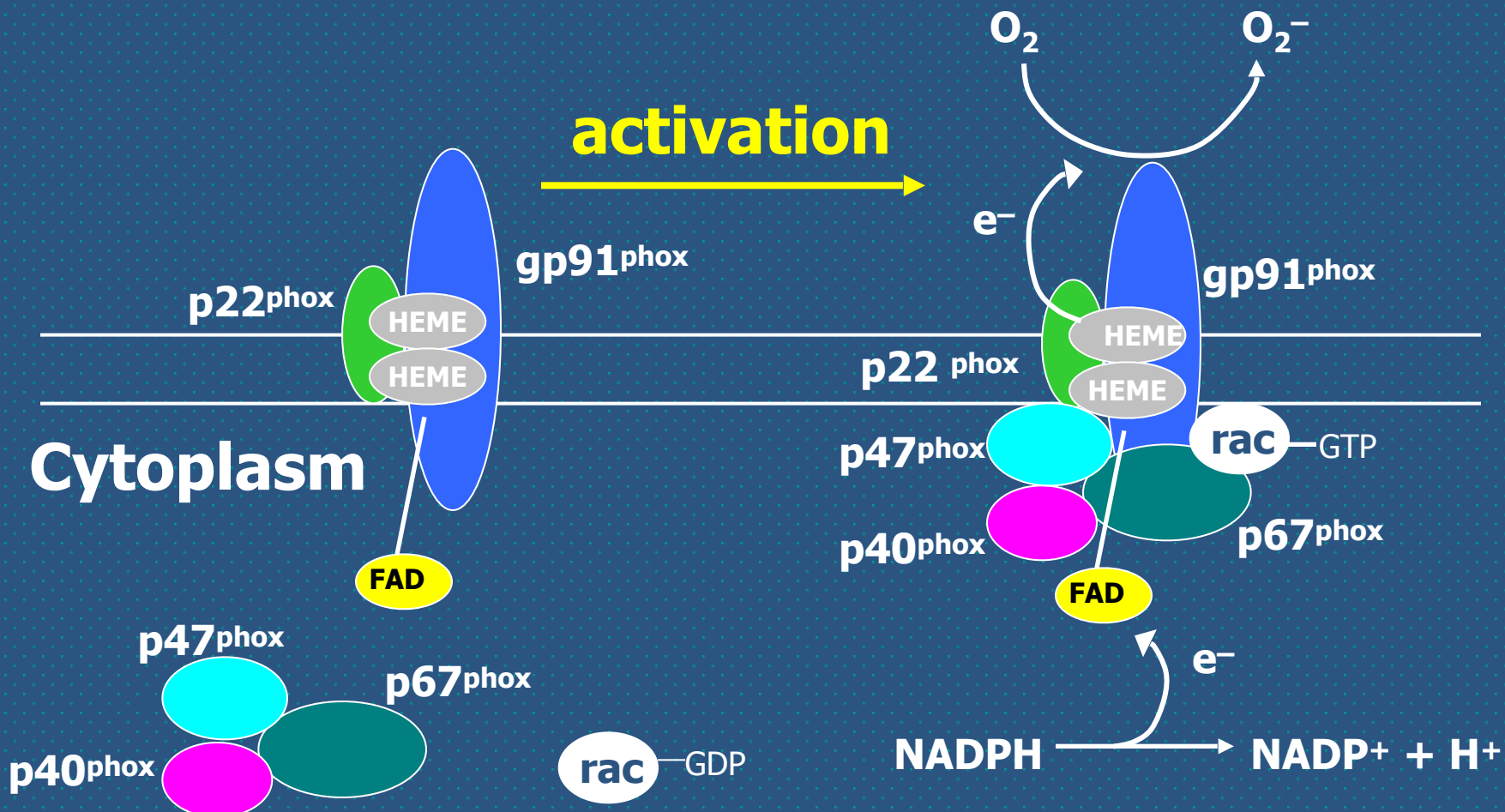
**KEY WORDS:** Chronic granulomatous disease, NADPH oxidase, Hematopoietic cell transplantation, Gene therapy

Chronic Granulomatous Disease (CGD): a disease of impaired antimicrobial host defense and excessive inflammation

# Chronic Granulomatous Disease

- CGD is a group of hereditary diseases caused by mutations of genes encoding phox proteins (**ph**agocyte **ox**idase, **phox**), which are the subunits of the ROS-forming phagocyte NADPH oxidase
- The phagocytes of CGD patients can ingest microbes but are unable of generating superoxide anion, hydrogen peroxide and other **ROS** that are crucial for antimicrobial activity
- CGD affects approximately 1 in 200,000 persons (North America and Europe)

# Phagocyte NADPH oxidase



# Molecular genetics of CGD

- Approximately **two-thirds** of CGD cases are **X-linked** (**gp91phox-deficient**), the **remainder** are **autosomal recessive** and are caused by mutations of genes coding for the subunits
  - p47phox (~ 20%)
  - p22phox (~ 6%)
  - p67phox (~ 6%)
  - p40phox (only one case)
- Patients with the **X-linked CGD** appear to be at **greater risk** for infection and early mortality **compared to patients with autosomal recessive forms**

# Main ROS forming reactions

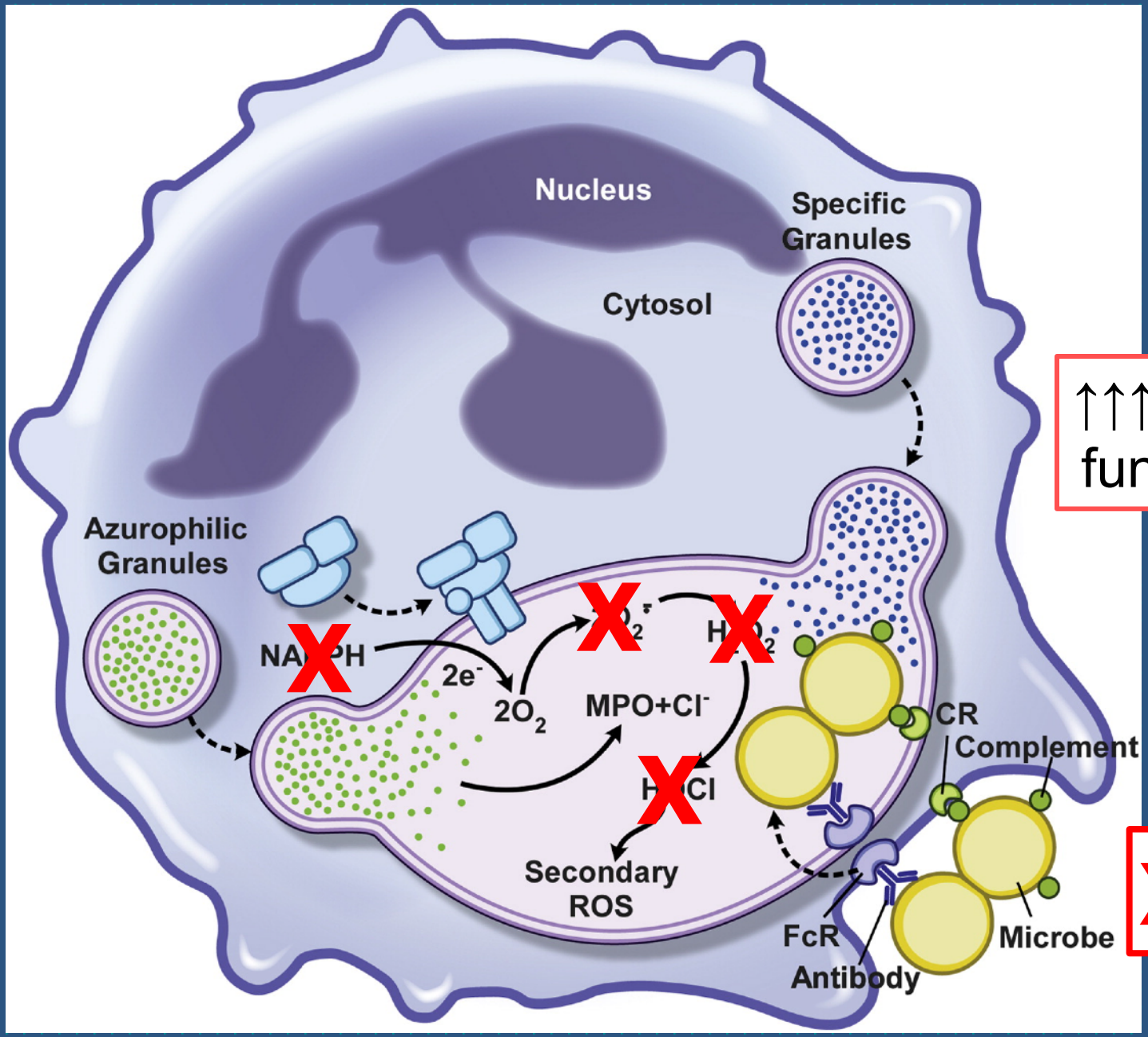
$$NADPH + 2\,O_2 \rightarrow NADP^+ + H^+ + 2\,O_2^{-\bullet}$$

$$2O_2^{-\bullet} + 2H^+ \xrightarrow{\text{superoxide dismutase}} O_2 + H_2O_2$$

$$H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} \mathbf{HOCl^-}$$

- The hydrogen peroxide-myeloperoxidase-halide sytem is considered the most powerful microbicidal mechanism acting inside the phagolysosome of neutrophils
- This system generates toxic molecules wich **oxidize** and **halogenate** vital constituents of phagocytosed microbes

# Microbicidal mechanisms of neutrophils

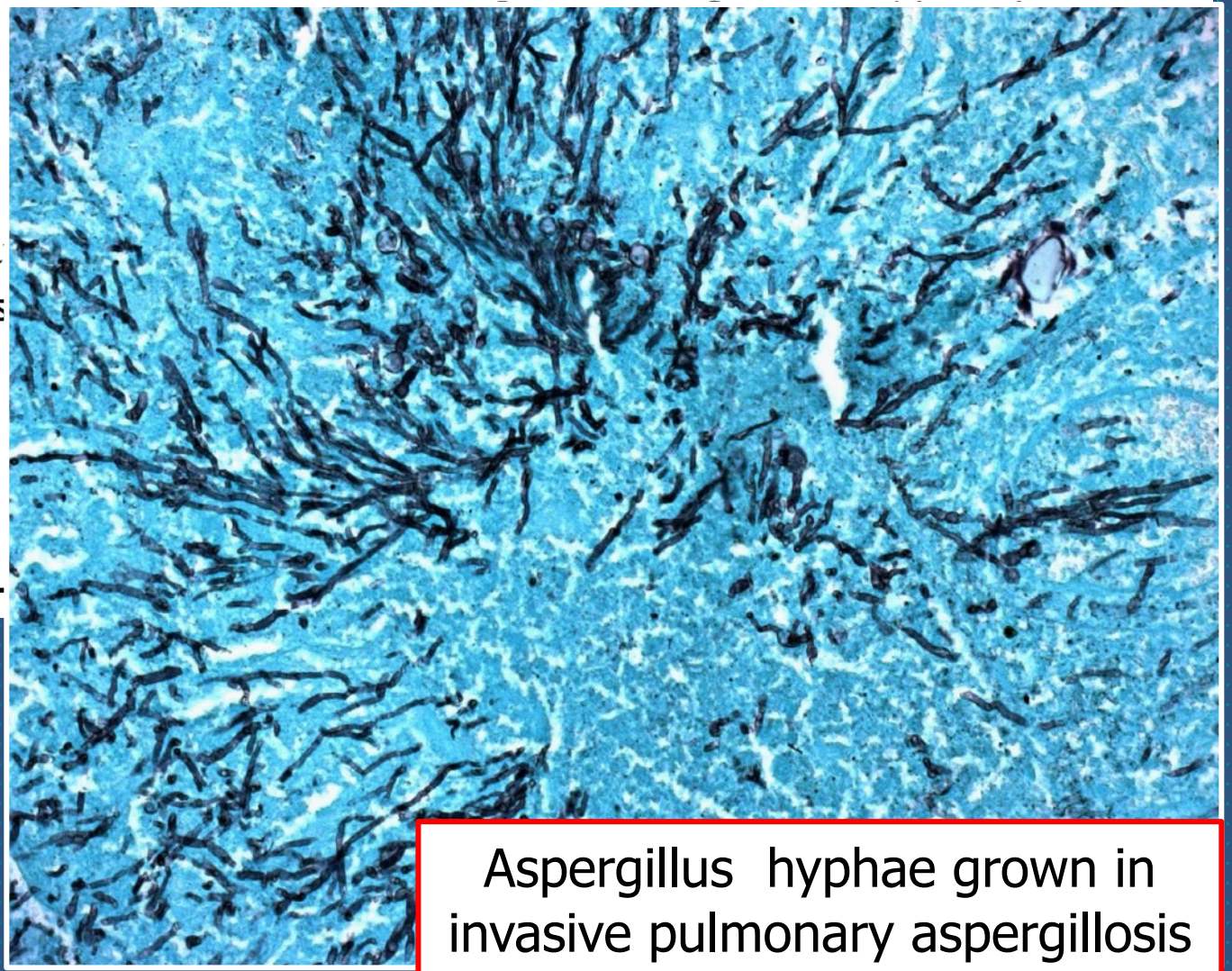



↑↑↑ bacterial and fungal infections

**X** in CGD

## Table 1. Infections in CGD

| Site | Most Common Pathogens |
|---|---|
| Lungs (pneumonia) | *Aspergillus* species and other molds, |
| Lymph nodes (suppura | |
| Skin (subcutaneous abs infected cysts) | |
| Liver (abscesses) | |
| Bone (osteomyelitis) | |
| Blood (sepsis) | |



Aspergillus hyphae grown in invasive pulmonary aspergillosis

# Chronic Granulomatous Disease: **treatment**

- CGD patients must receive **antibacterial** and **antifungal prophylaxis**, the latter beeing very aggressive
- **Recombinant interferon-γ** significantly reduces the incidence of serious infections in CGD patients likely because this cyokine potentiates **oxidant-independent** microbicidal mechanisms (granular microbicidal proteins)
- **Hematopoietic cell transplantation** with HLA identical sibling or closely matched unrelated donor (≥ 7/8 HLA antigens) is a valid therapeutic option early in life

# Chronic Granulomatous Disease: **treatment**

- **Gene therapy**: most of the current gene therapy research focus on lentivirus vectors

- Lentiviral infection have advantages over other gene therapy methods including:
  - high-efficiency infection of dividing and non-dividing cells
  - long-term stable expression of a transgene
  - low immunogenicity

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# Chediak-Higashi Syndrome (CHS)

- Rare genetic disorder which affects many organs, and particularly the immune system

- In CHS, mutations in the LYST gene result in abnormal function of the **lysosomal trafficking regulator** protein which affect the size and function of lysosomes

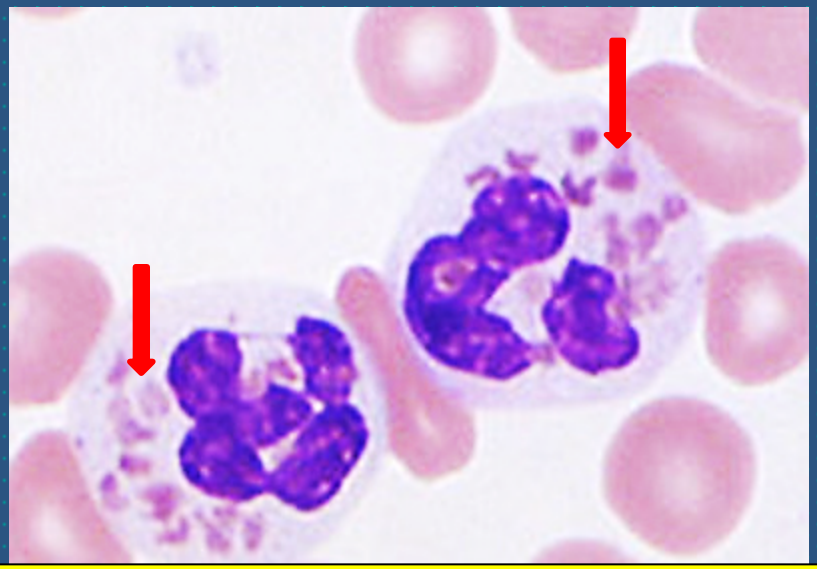
- The defects result in the progression of viral and bacterial infections starting in infancy or early childhood which are in some cases lifethreatening: few people with this condition can live to adulthood

# Chediak-Higashi Syndrome

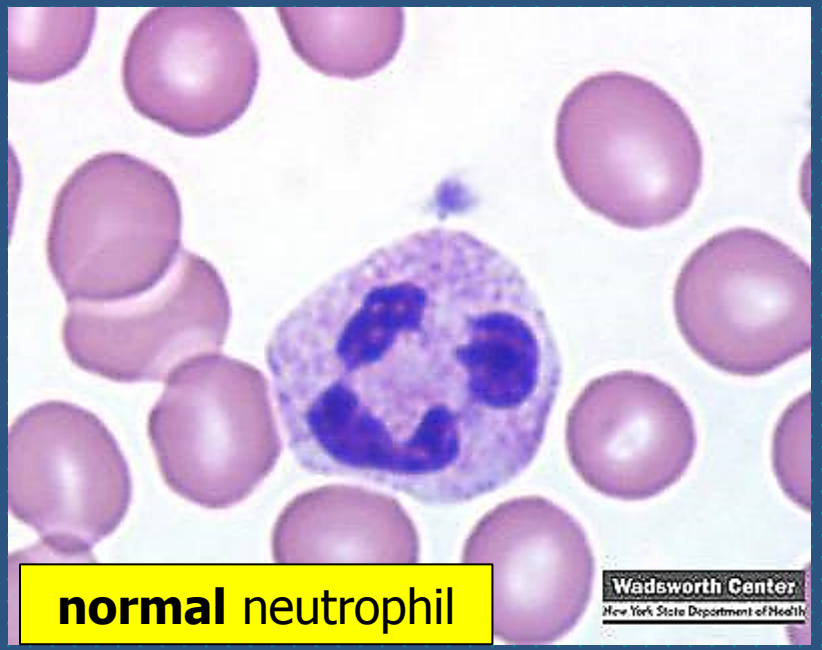
- Abnormal large granules in a variety of cells lead to:
- **Hypopigmentation/partial albinism**: impaired packaging of melanin into giant melanosome granules disturb melanin traffic
- **Severe immunodeficiency**: impaired release of lytic secretory granules by NK cell; neutrophil defects (see next slide)
- **Neurologic abnormalities**: cytoplasmic inclusions, resembling large lysosomes, were present in all types of neurons; peripheral and cranial neuropathy
- **Mild bleeding tendencies**: absent or reduced number and irregular morphology of platelet-dense bodies (required for the secondary wave of platelet aggregation)

# Immunodeficiency in CHS

- Giant granules result from abnormal fusion of primary granule (azurophilic) with secondary granule (specific)
- The presence of giant granules in neutrophils impairs chemotaxis and killing of ingested microbes



**CHS** neutrophil (arrows point to giant granules)




**normal** neutrophil

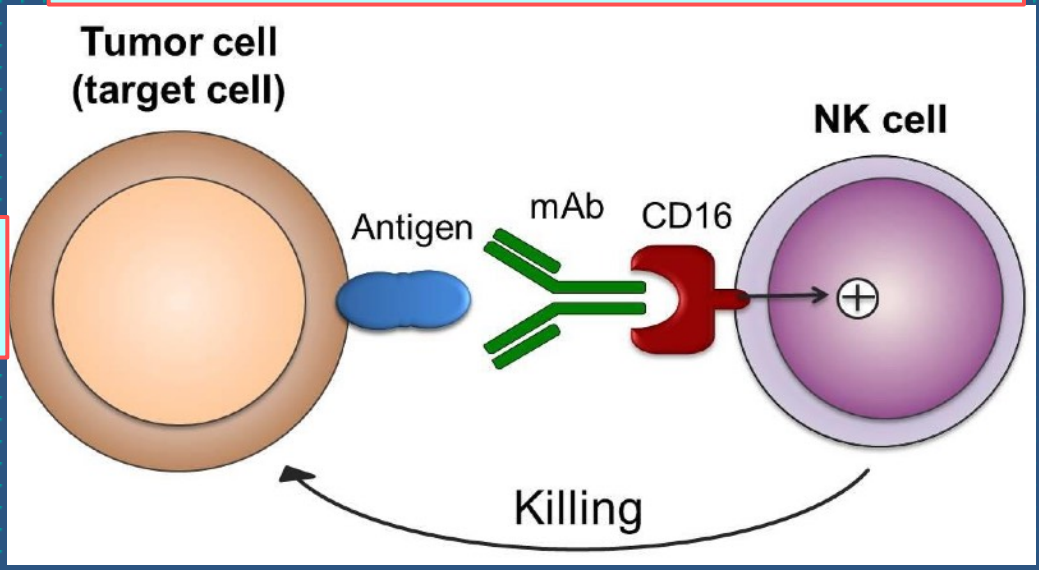
# NK cells

NK cells are best appreciated for innate defense against **viral infections** and in **tumor cell surveillance**; they participate also in the **regulation of immune response** (release of cytokines: TNF, IL-10, GM-CSF, IFN-γ)

**Mechanism of NK cell recognition of normal cells (A) and target cells such as tumor or virally infected cells (B).** When NK cells recognize self, normal cells, the interaction of the **inhibitory receptor** with cognate MHC I molecules **blocks killing activity**.

**Lack** of self MHC I molecules **on target cells**, triggers NK cells' **cytotoxicity** release of **cytotoxic molecules** (e.g. **perforin** and **granzymes**)

**Antibody dependent cell mediated cytotoxicity (ADCC)**



# NK deficiency

- NK cell deficiency (NKD) represents a small but increasingly appreciated subset of primary immunodeficiency diseases
- Mutations of genes involved in NK **maturation** and **functions** are responsible for such deficiencies
- Commonly, patients with NKD have increased susceptibility to Herpes viruses and other viral pathogens
- Up to know, direct proofs showing an increased susceptibility of NKD patients to cancer development are lacking

# NK deficiency

- NKD can be divided into two major types:
  - **classical NKD** (CNKD) is defined as an absence of NK cells among peripheral blood lymphocytes
  - **functional NKD** (FNKD) is defined as the presence of NK cells within peripheral blood lymphocytes, having defective NK cell activity

- In both CNKD and FNKD the NK cell abnormality is the major immunological deficit resulting in inadequate host defense