

Innate immunity

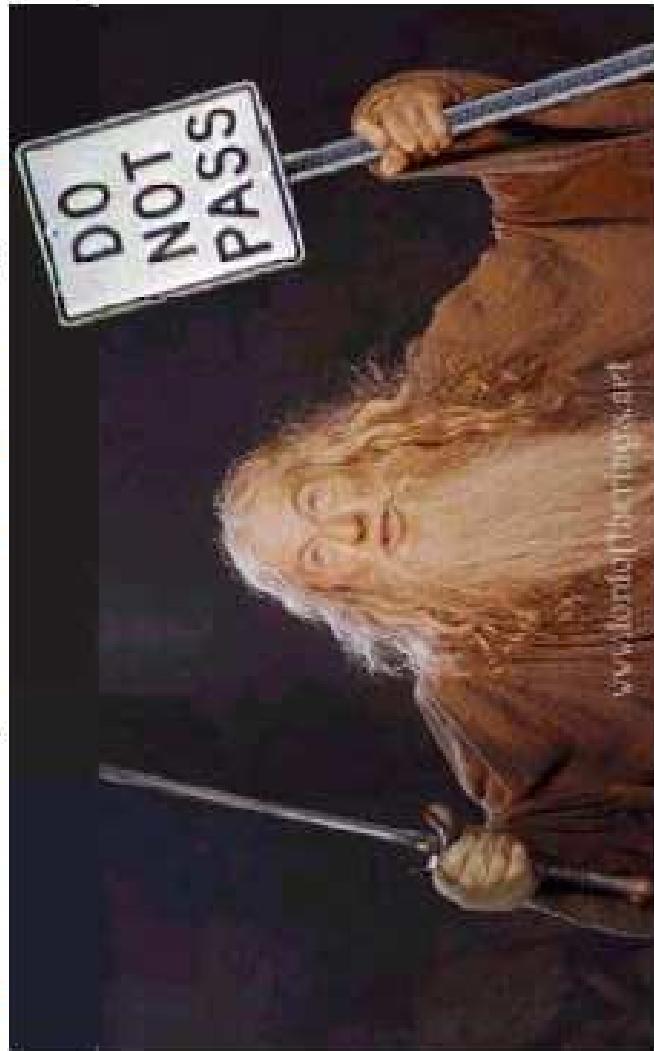
learning objectives

B. Innate immunity

- innate immune cell types and their functions.
- Gram positive and gram negative bacteria
- PAMP, PRR and TLR
- NK, HLA and KIR

What is the function of the innate immune response?

The main function of the innate immune system is to clear infections before they become harmful or systemic.



Why do we need Innate Immunity?

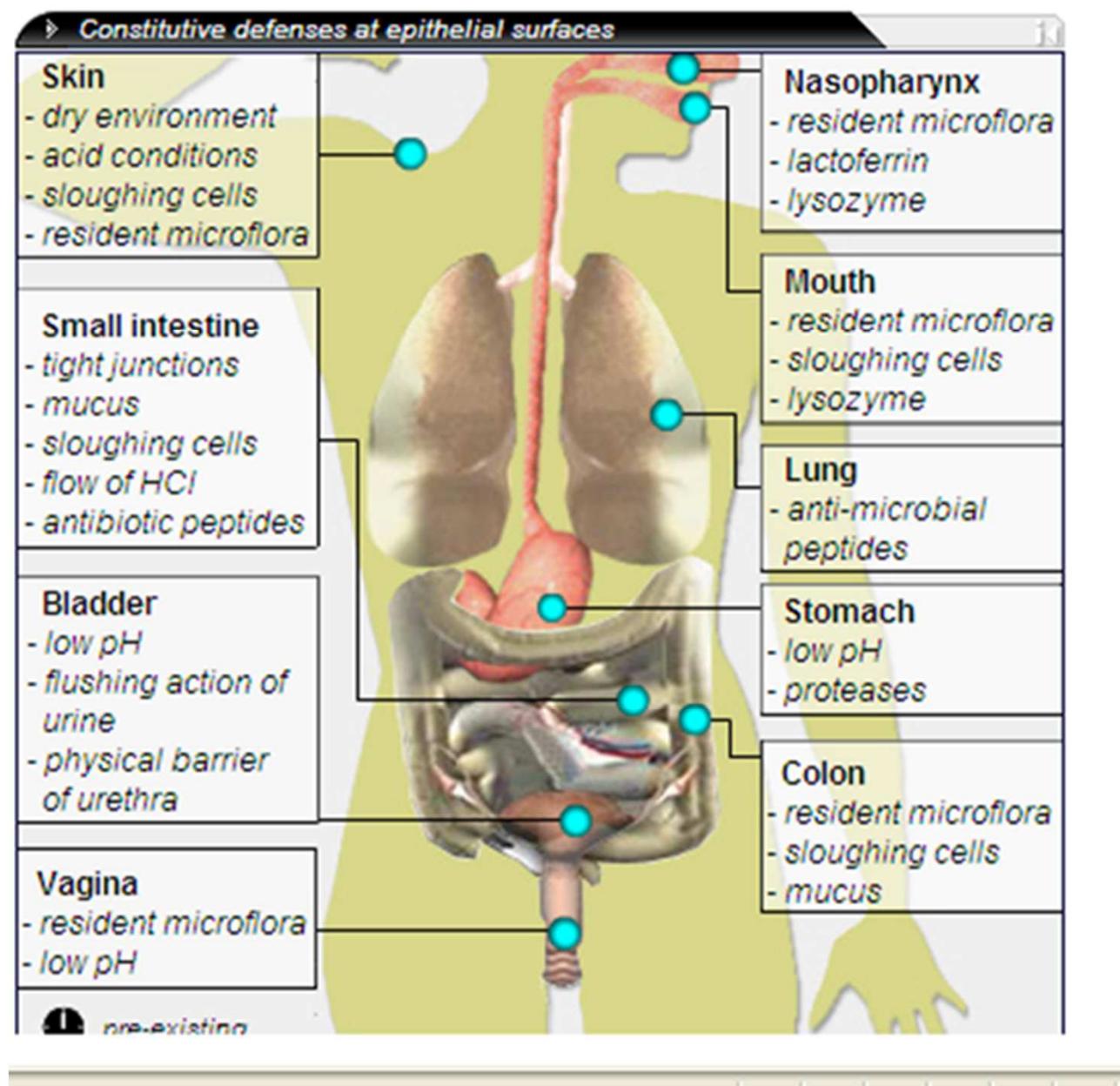
- Pathogens multiply very fast
- E. coli doubles every 30 minutes= 2×10^{143} bugs in 3 days
- But adaptive immunity requires 3-14 days to respond (T and B cells action)

Innate immune system - part 1: barriers

These include many tissues and organs

- Anatomical, mechanical, chemical and microbial barriers
- Skin: a physical barrier, low pH
- mucous membranes lining the digestive, respiratory and reproductive tract
- Hair – on skin , nose, eyelashes,
- Secretions e.g. tears, sweat, mucus, saliva; lysozyme
Microbiome: existing bacteria living on your skin block others from entering
- Stomach – low pH, enzymes

Innate immune system - part 1: barriers

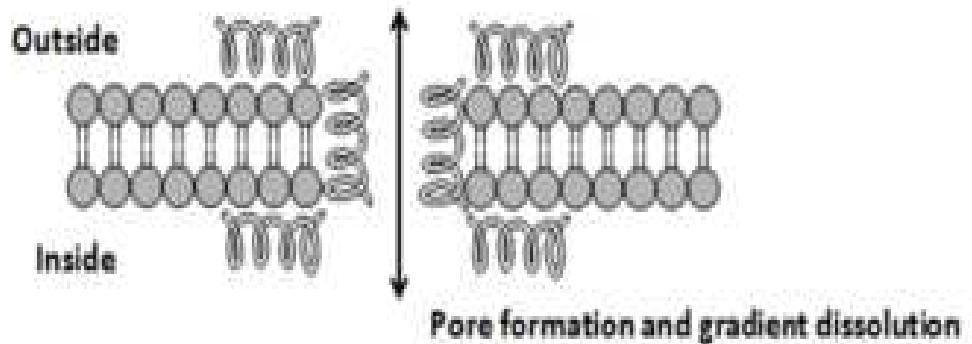


AMPs

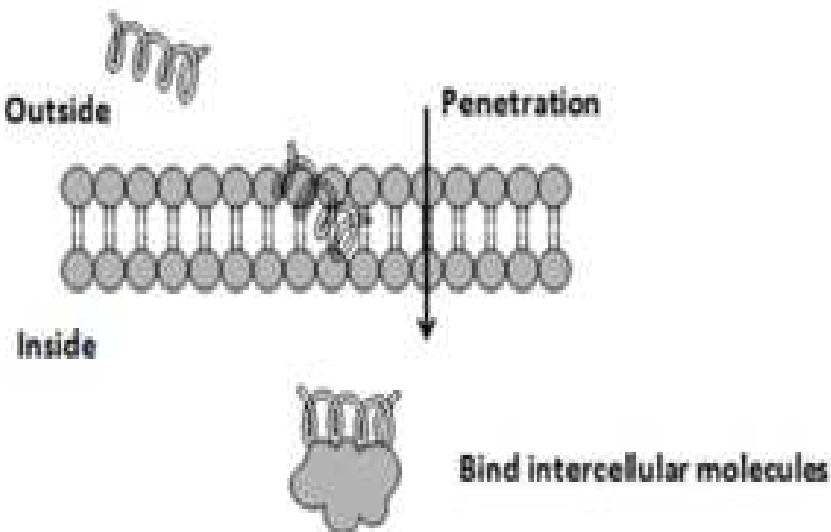
Antimicrobial peptides

- short amino acid chains
- 12-50 aa long
- include two or more positively charged residues and >50% hydrophobic residues
- act against microbes and pathogens
- Varied modes of action e.g. **form pores in target cell membrane** or bind to intracellular molecules
- E.g. defensins from many species, antibiotics from bacteria, lysozyme from humans, quercetin from plants, etc
- Therapeutic potential

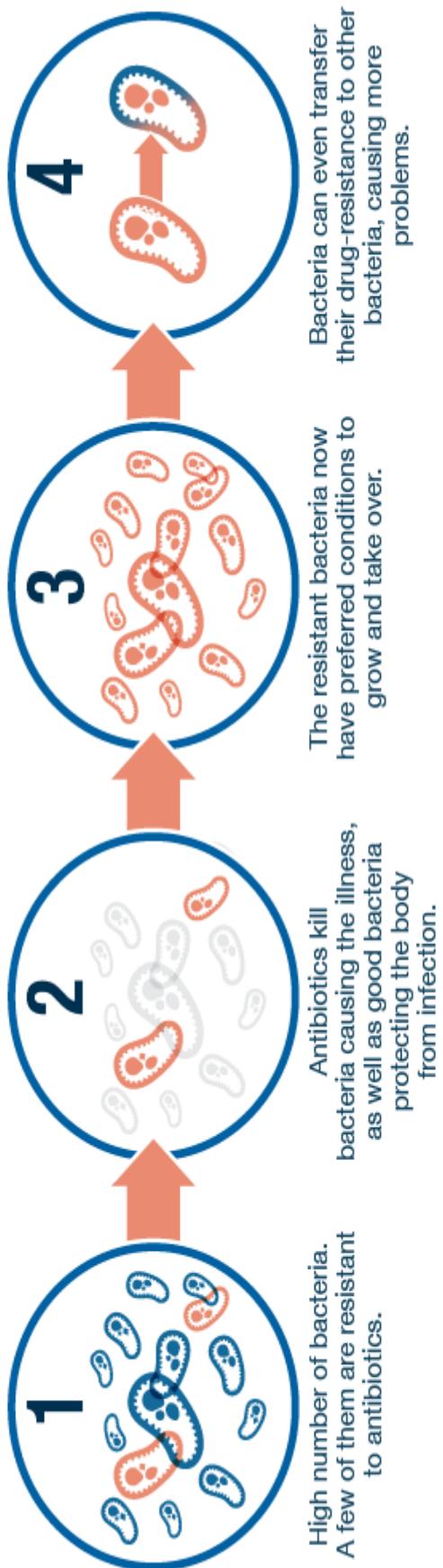
Transmembrane pore-forming



Modes of intracellular killing



How does antibiotic resistance occur?

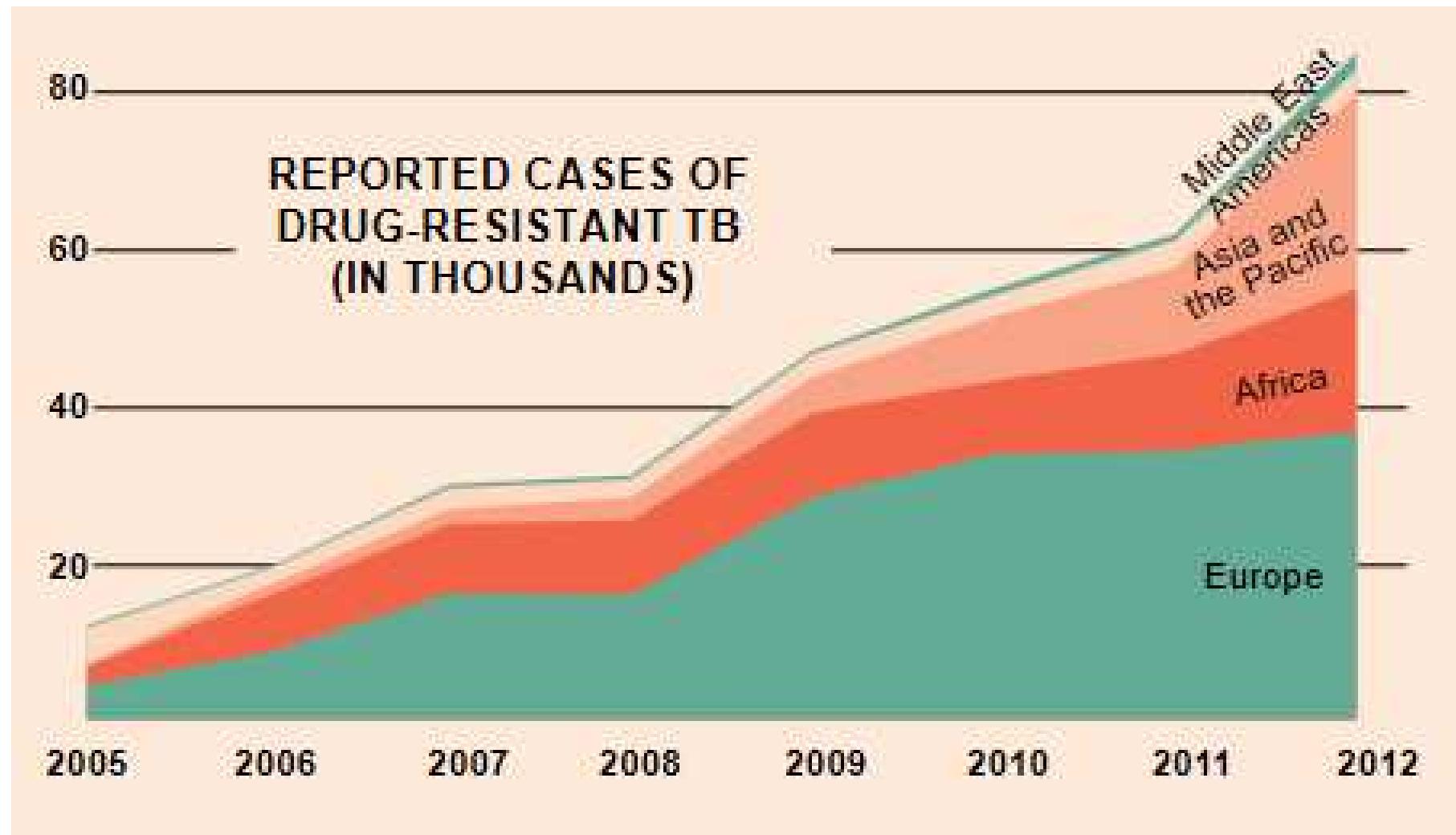


CAUSES OF ANTIBIOTIC RESISTANCE



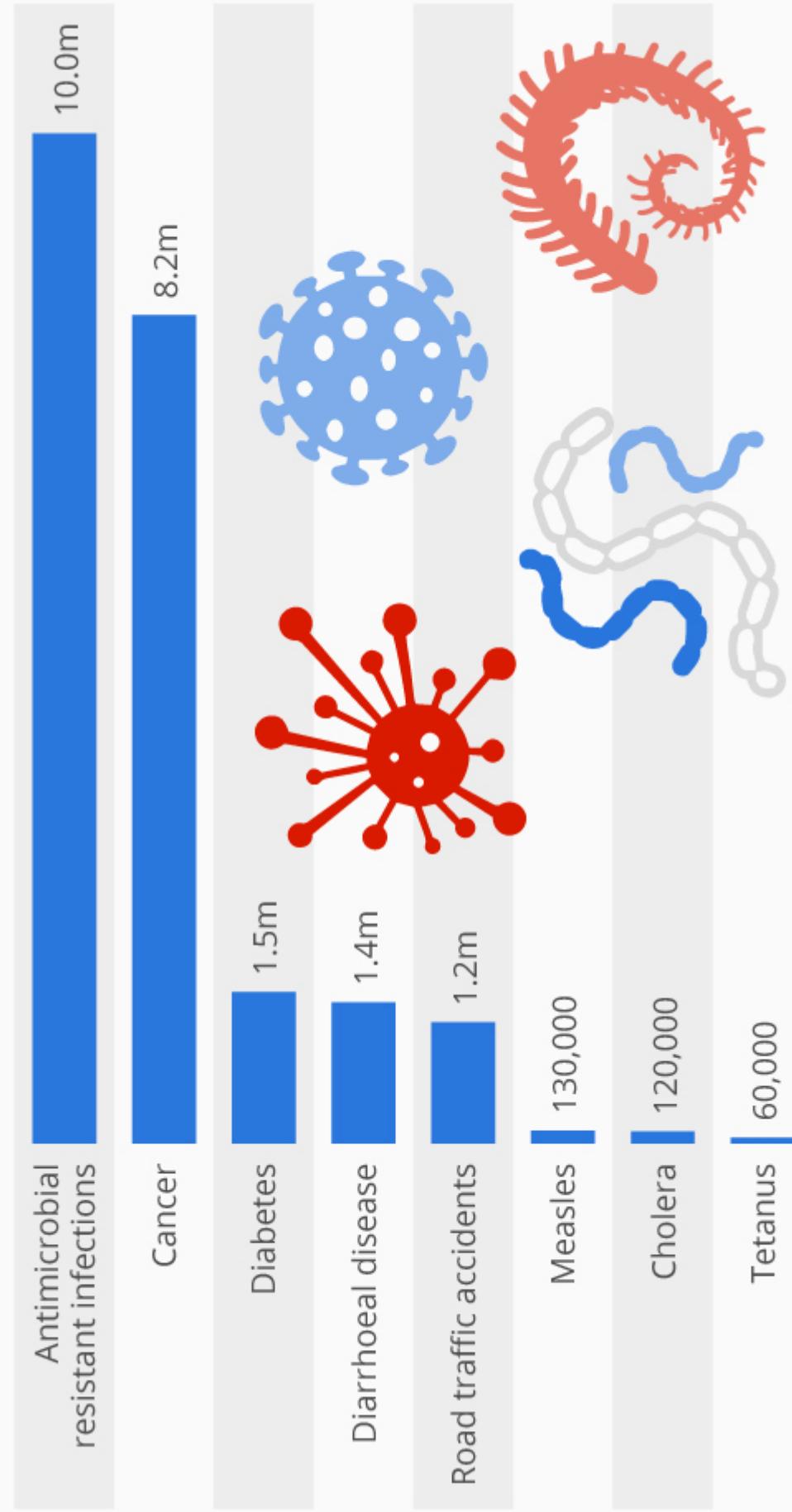
WHO/CDR/Resistance
www.who.int/drugresistance

Antibiotic resistance is a growing problem



Deaths From Drug-Resistant Infections Set To Skyrocket

Deaths from antimicrobial resistant infections and other causes in 2050



Innate immune system - part 2: cells

Cells of the innate immune system include

1. Granulocytes (Neutrophils, basophils, eosinophils)
2. Monocytes (macrophages ,dendritic cells)
3. Natural killer cells

Cell functions in innate immunity

Cells of the innate immune system include

1. Granulocytes (Neutrophils, basophils, eosinophils)
2. Monocytes (macrophages ,dendritic cells)
3. Natural killer cells

Do you remember the major functions of these cells?

- Most innate cells (except NK) perform **phagocytosis** and produce **antimicrobial peptides**
- All immune cells produce **cytokines**
- Some specialised cells (macrophage and DC) perform **antigen presentation**
- Some of the granules produced by granulocytes contain **antimicrobial peptides** or are **cytotoxic** (neutrophils, eosinophils); NK are also cytotoxic
- Activate **complement**
- **Inflammation**

Activity: match these functions to their correct cell type:

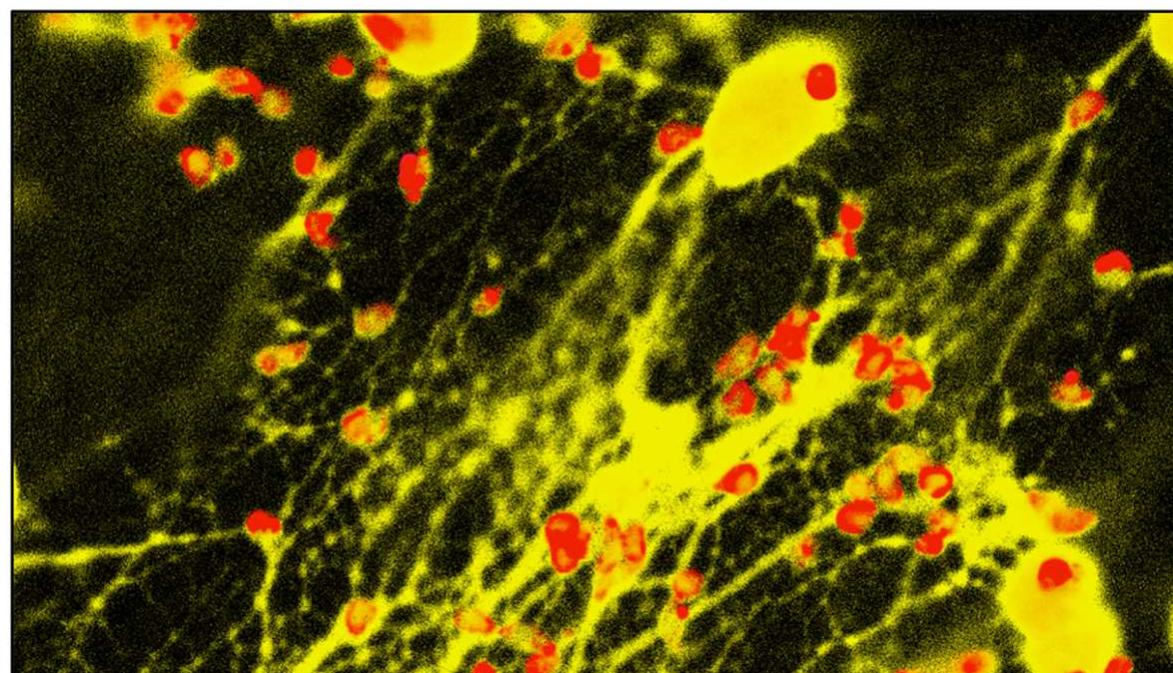
Cytolysis; antigen presentation; cytokines; AMP; phagocytosis

Innate Cell type	Function
Granulocytes Neutrophils Basophils eosinophils	
Monocytes Macrophages dendritic cells	
Natural killer cells	

cells	Cell types	• Function	Particular targets
Lymphocyte	NK	<ul style="list-style-type: none"> • Cytolysis • cytokines 	Cancer cells Virally infected cells
granulocytes	Neutrophils	<ul style="list-style-type: none"> • Phagocytosis • cytosis • NETs 	bacteria
	basophils	<ul style="list-style-type: none"> • inflammation 	
	eosinophils	<ul style="list-style-type: none"> • Cytolysis, • phagocytosis • Allergy 	parasites
Monocytes	macrophages	<ul style="list-style-type: none"> • Phagocytosis • Antigen presentation • cytokines 	Viral infected cells bacteria
	Dendritic cells	<ul style="list-style-type: none"> • Phagocytosis • Antigen presentation • Cytokines 	

NETs

- Neutrophils can throw out webs of their own DNA to create “NETs” =Neutrophil extracellular traps.
- The web of chromatin fibres trap bacteria or other extracellular pathogens and also help to concentrate antimicrobial compounds; may help with blood clotting



Check your understanding

1. Give some examples of physical barriers of the innate immune system.
2. Why is the development of AMPs as therapeutic products important?
3. Give some examples of cells of the innate immune system and their major functions.

Innate immune system - part 3: receptors

Q: how do innate cells recognise pathogens ?

Reminder:

	INNATE
What it recognises	Not specific – recognises pathogen patterns
Receptors	Small set / limited variety
How fast it reacts	Immediate responses (hours)
Memory?	no
In which species it is found	All vertebrates
Cells and tissues	<u>Barriers</u> : Anatomical and physiological, inflammation <u>Cells</u> : phagocytes, Nk cells <u>Humoral</u> : complement, antimicrobial peptides

Q: how do immune cells recognise pathogens ?

There are billions of different microbes in the world....

How does the immune system know which are pathogens?
How does the immune system recognise that these are not
“self” or “host”?

This is like asking: there are billions of people in the world.
How would you go about recognising people in a crowd?
Who is “safe ” or known” ? Who is strange or unknown?

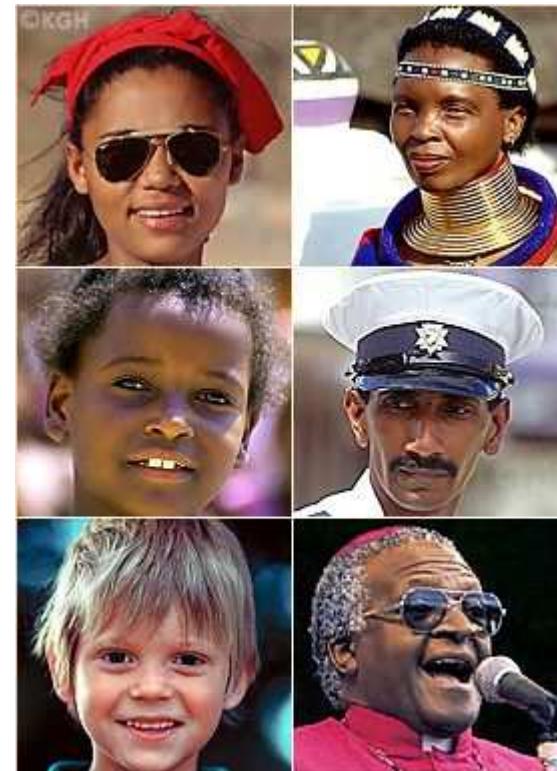


2 approaches to person recognition

Look for a pattern to identify people you know



Look for particular individual faces you know



2 approaches to pathogen recognition

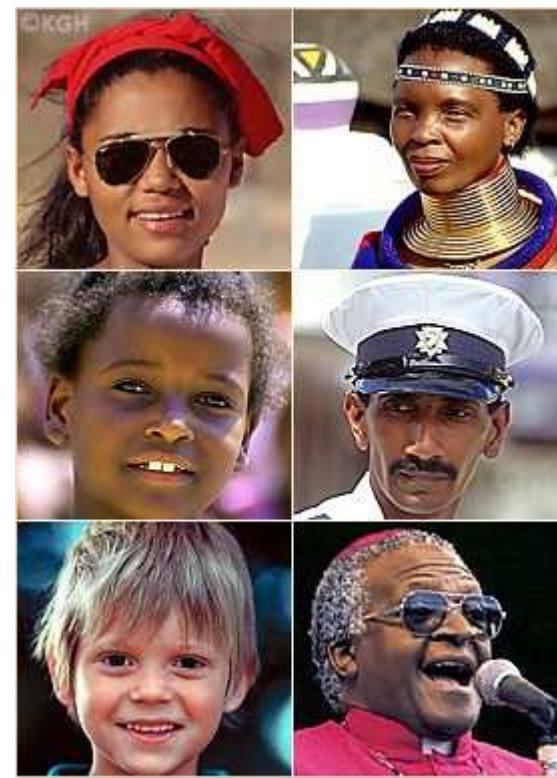
INNATE

Look for a pattern



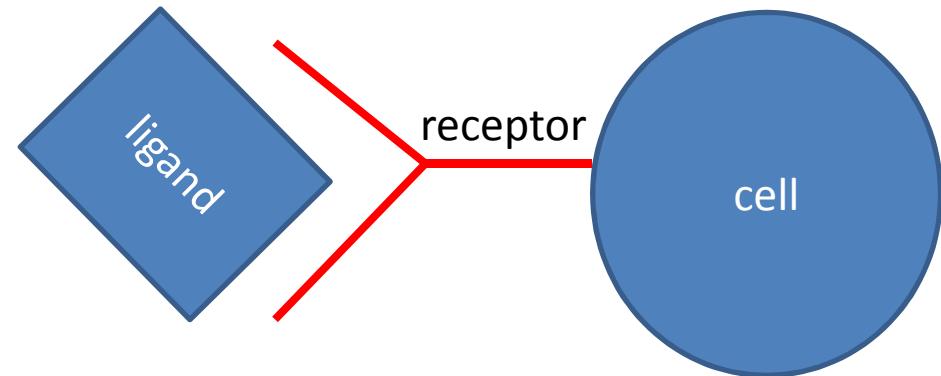
ADAPTIVE

Look for particular details



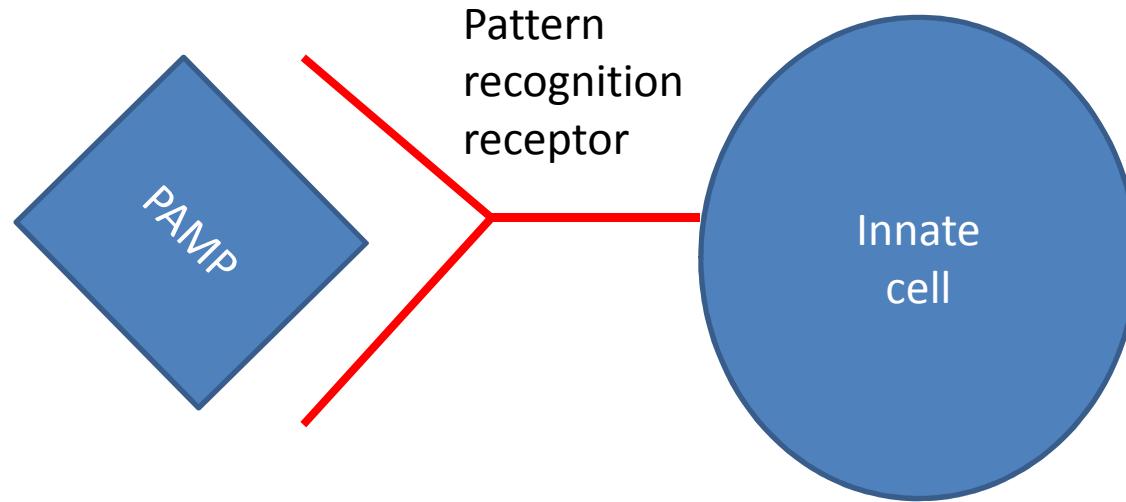
Q: what patterns do innate cells recognise ?

- “keys and locks” of cell recognition
- Many cells in the body have receptors that respond to particular substances known as ligands
- Has to be the right “fit” in order for the cell to respond to that ligand



- The innate cells have receptors on their cell surfaces
- that recognise particular patterns of substances produced by pathogens

Q: how do innate cells recognise pathogens ?



- The innate cells have receptors on their cell surfaces known as Pattern Recognition Receptors (PRRs)
- that recognise particular patterns of substances produced by pathogens , known as Pathogen-Associated Molecular Patterns (PAMPs)

which microbial patterns (PAMPs) are recognized?

- Components of bacterial cell wall e.g.
peptidoglycan, LPS
- Bacterial flagella
- Bacterial DNA and RNA
- Viral DNA and RNA
- Fungus cell wall (glucan)

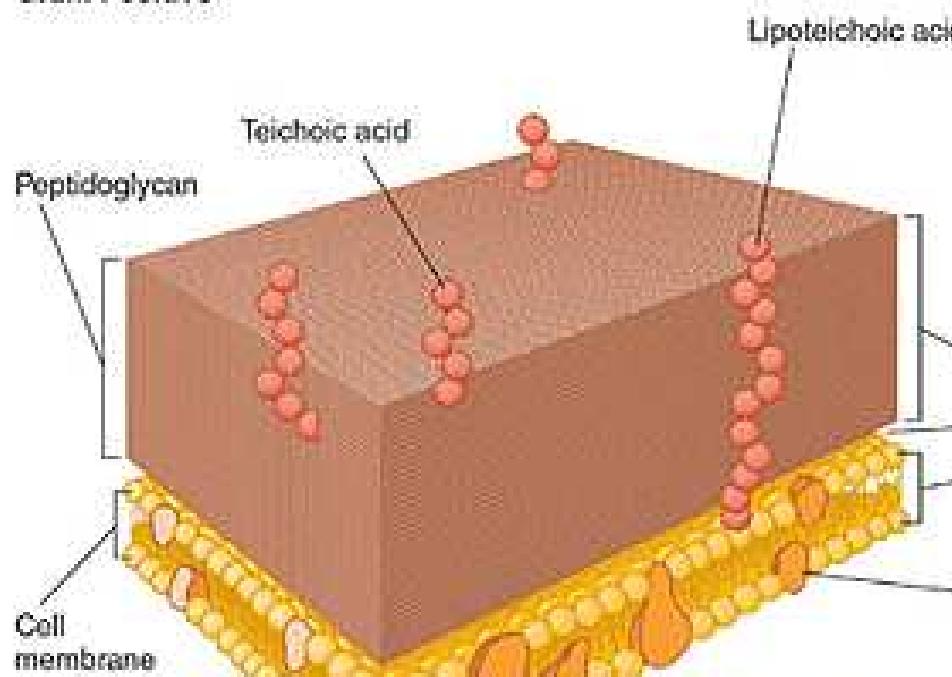
Bacteria cell walls

Thick cell wall

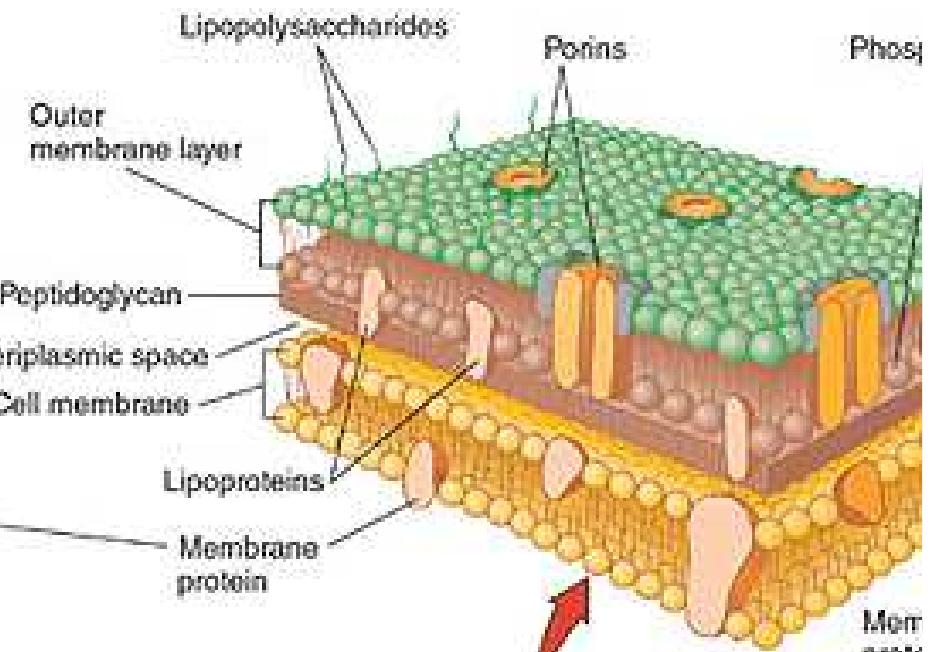
Thin cell wall
plus outer
membrane

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Gram Positive



Gram Negative

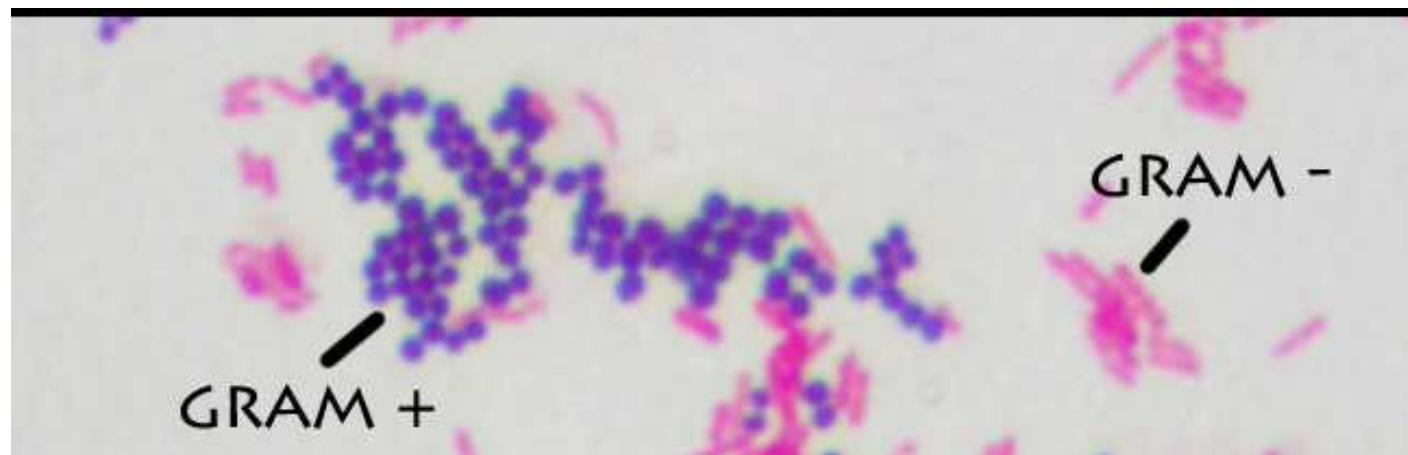


- Thick Cell wall
- Inner membrane

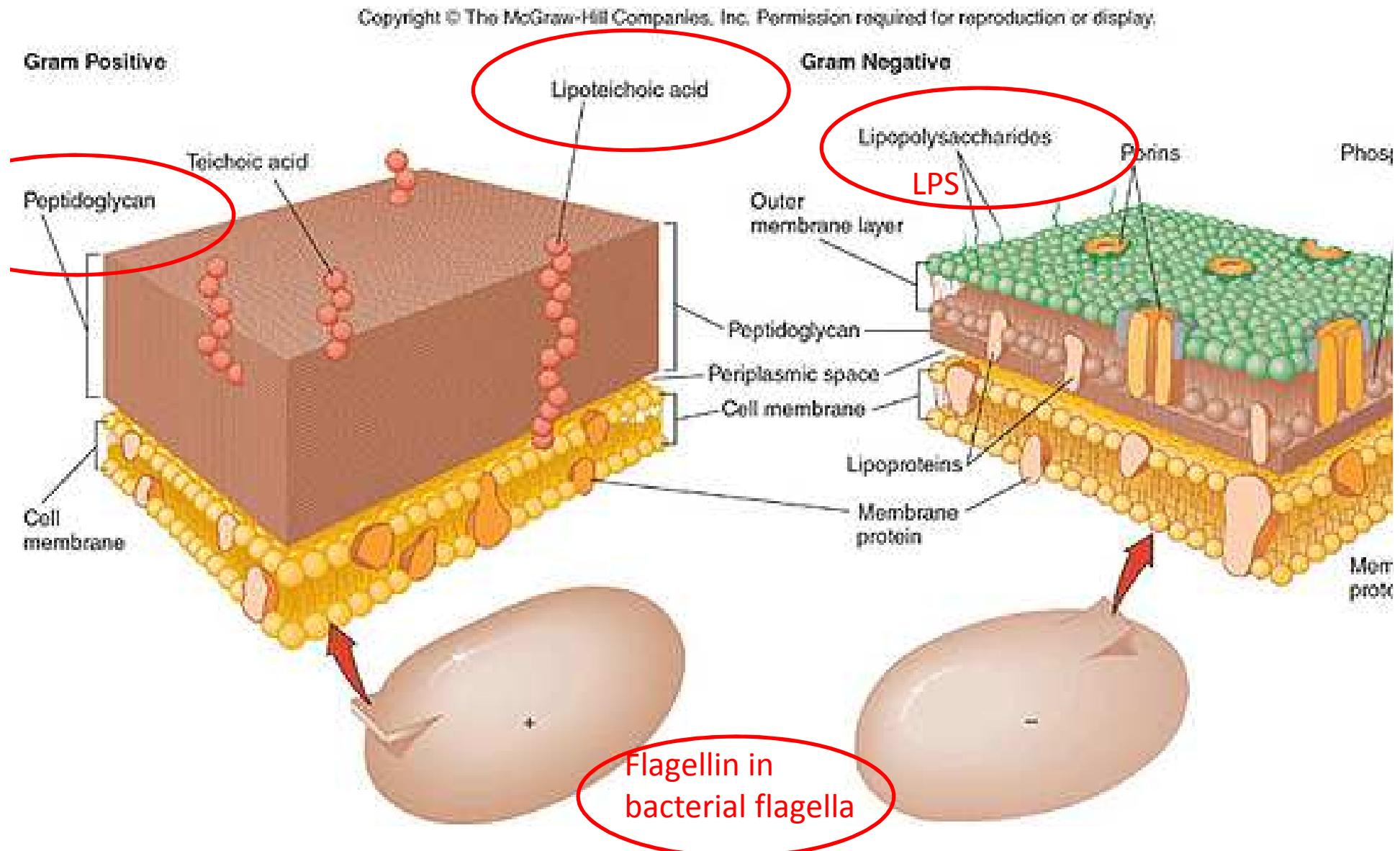
- LPS
- Outer membrane
- Thin Cell wall
- Inner membrane

Gram staining shows 2 large classes of bacteria

- Gram staining means add 2 dyes: crystal violet dye and a pink dye such as safranin
- bacteria will either stain purple or pink depending on the thickness and composition of their cell wall
- **Bacteria with thick cell walls containing peptidoglycan stain purple**
- **Bacteria with thin cell walls and little peptidoglycan stain pink**
- (prac later this block)

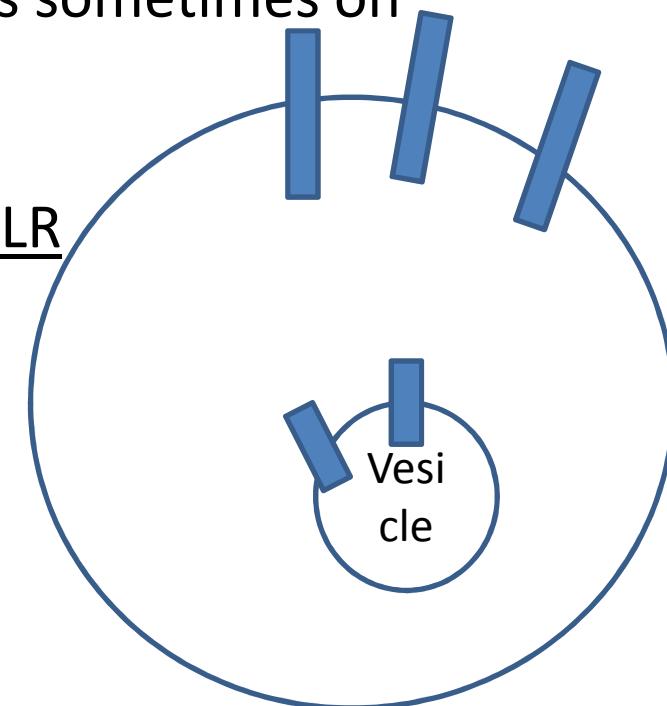


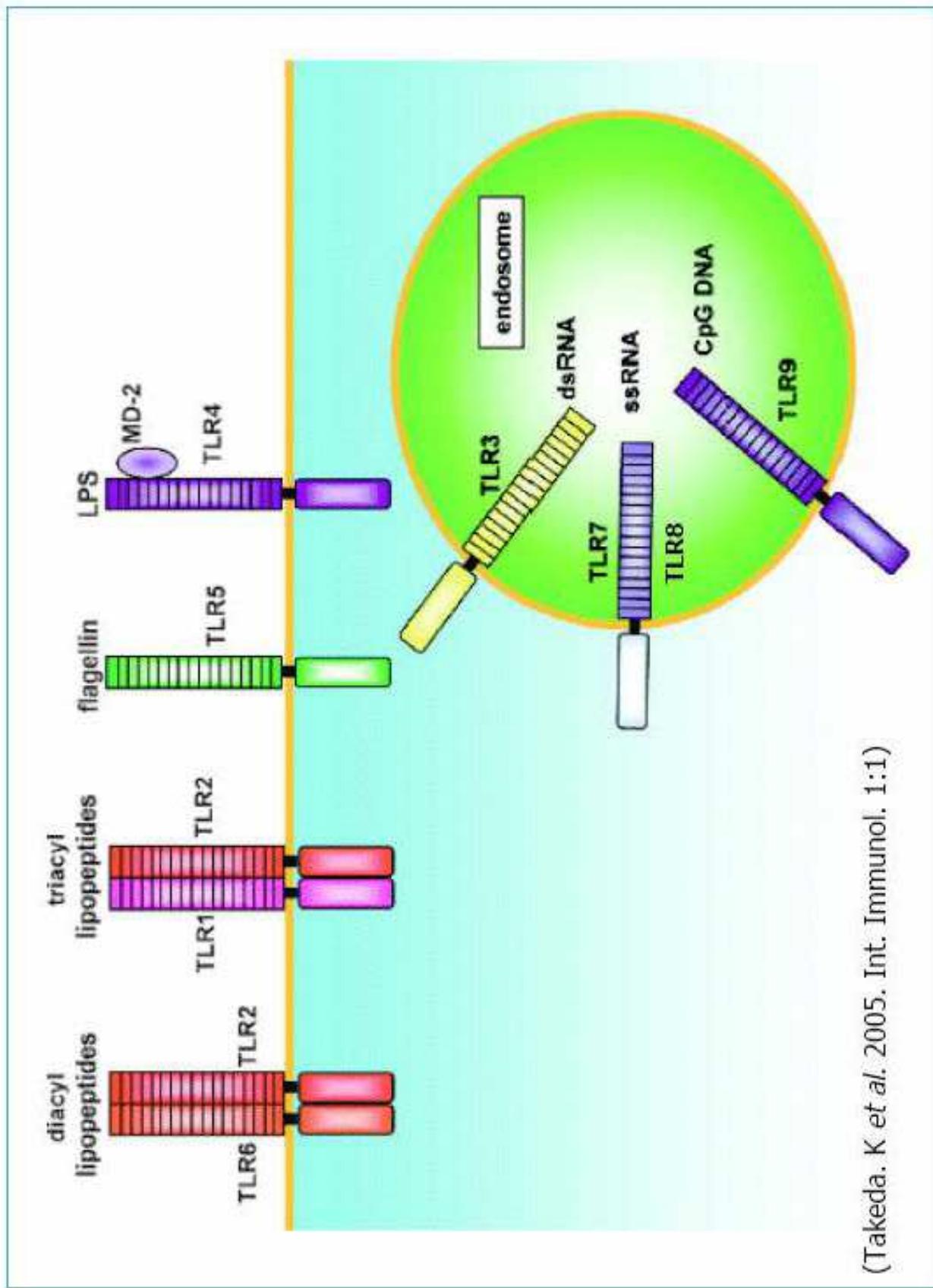
Components of the cell walls are recognised by the innate immune system



Q: how do innate cells recognise pathogens ?

- One important family of PRR are called the Toll Like Receptors (TLRs)
- There are up to 10 different types of TLR that recognise different PAMPs
- TLRs are found on innate cells like macrophages and DC (also on B cells)
- TLR can be on the surface of cells, as well as sometimes on membranes inside cells
- Different PAMPS for external and internal TLR
- Such as

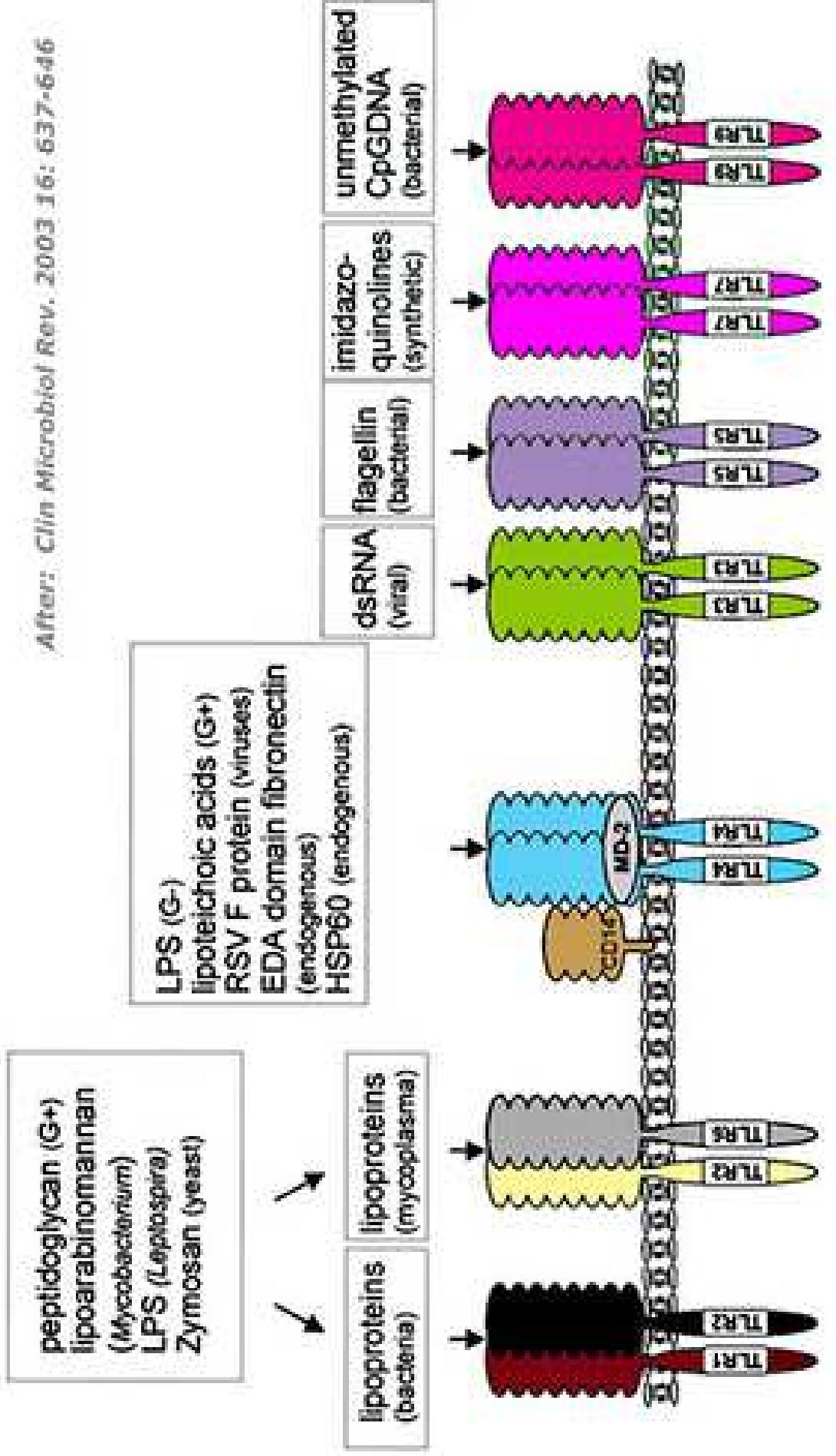


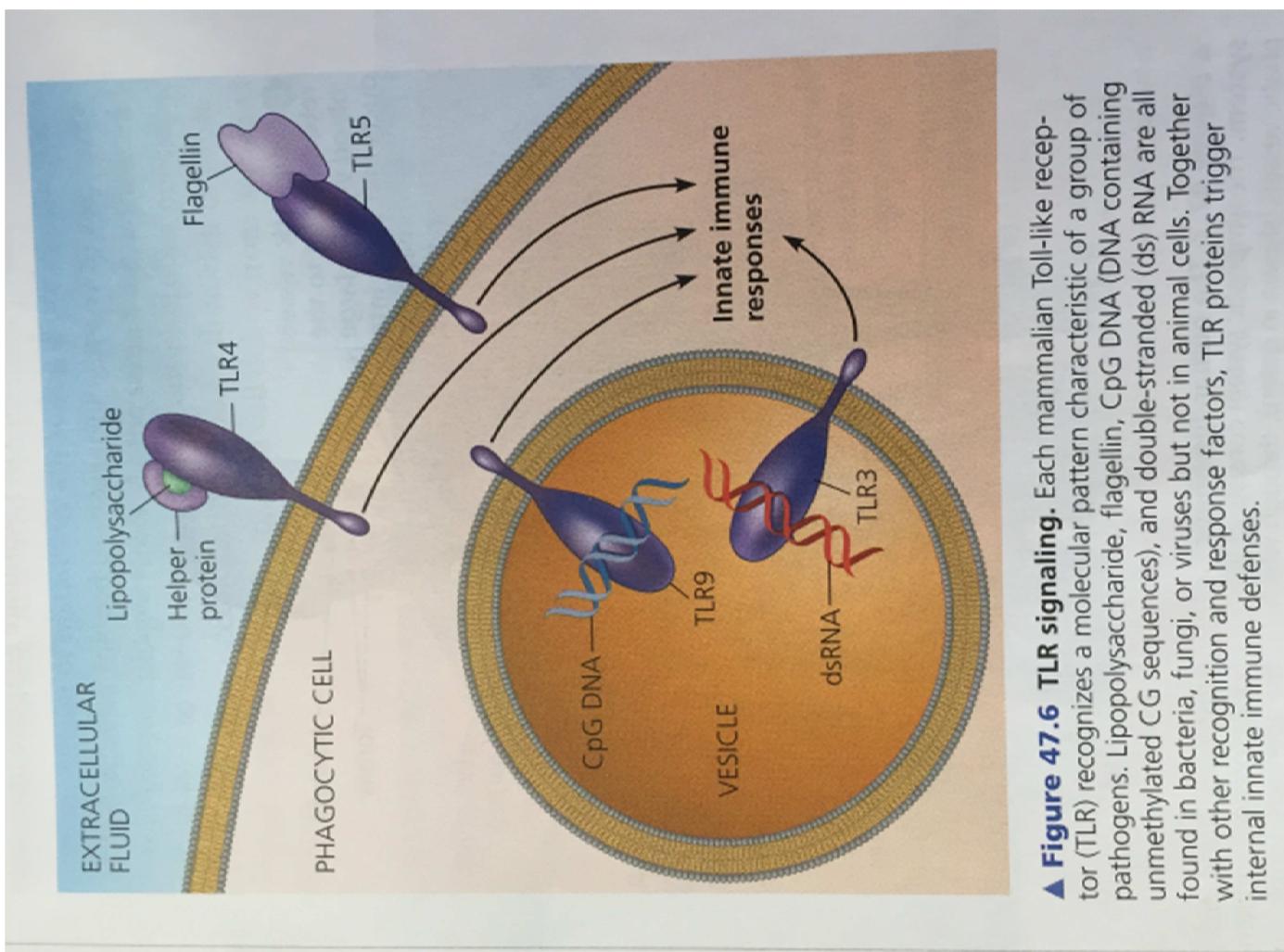


examples of TLR-PAMP pairs

TLR type	TLR location	PAMP	Source of PAMP
2	surface	lipoteichoic acid; peptidoglycan	Gram positive bacteria
4	Surface	Lipopolysaccharide (LPS)	Gram negative Bacteria
5	surface	Flagellin	Bacteria
3	Internal	Double stranded RNA	Viruses
9	Internal	Unmethylated CpG DNA	Bacteria or viruses

After: *Clin Microbiol Rev*, 2003 16: 637-646



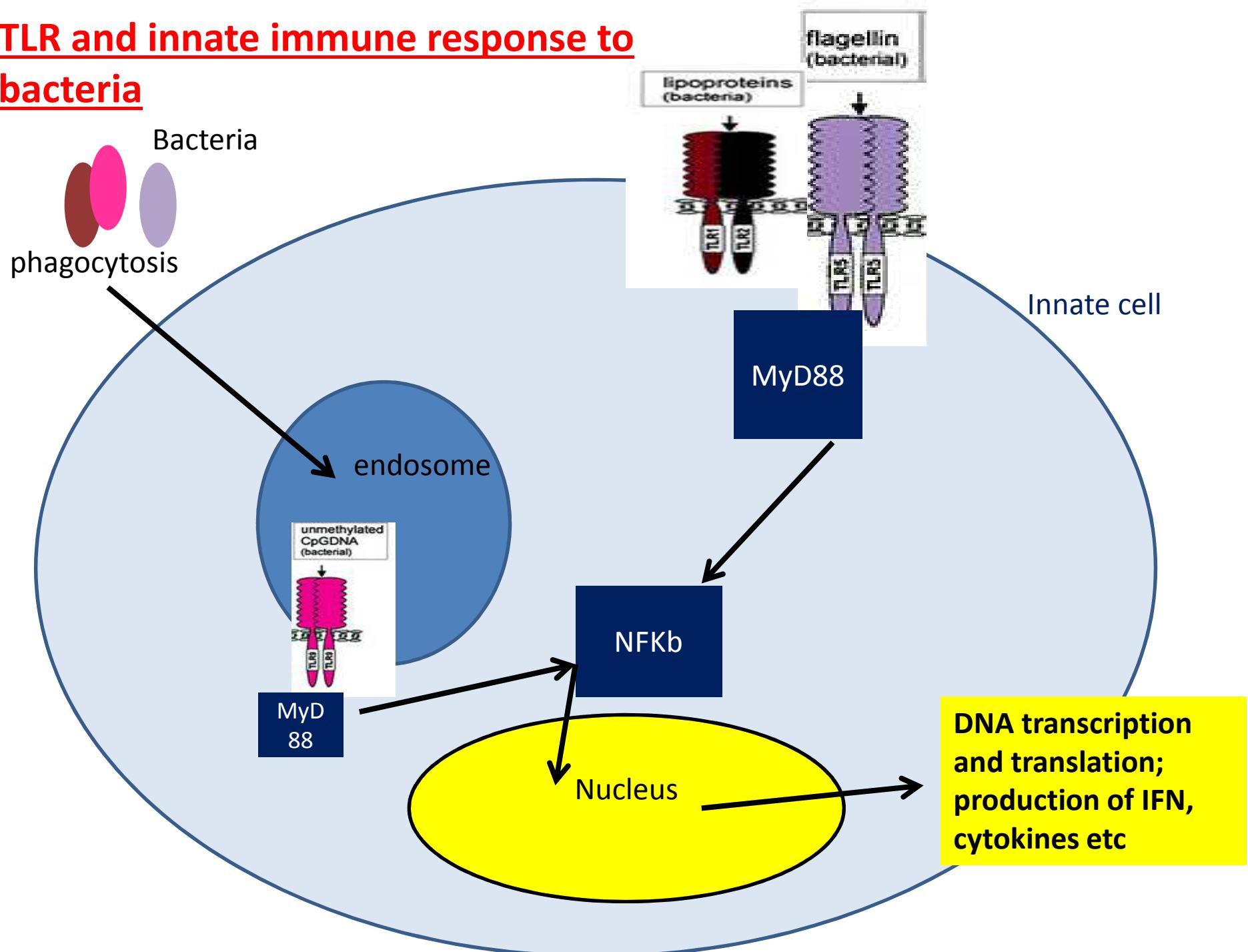


▲ **Figure 47.6 TLR signaling.** Each mammalian Toll-like receptor (TLR) recognizes a molecular pattern characteristic of a group of pathogens. Lipopolysaccharide, flagellin, CpG DNA (DNA containing unmethylated CG sequences), and double-stranded (ds) RNA are all found in bacteria, fungi, or viruses but not in animal cells. Together with other recognition and response factors, TLR proteins trigger internal innate immune defenses.

What does TLR-PAMP signalling do?

- TLR- PAMP binding triggers a response in innate cells
- two different signalling pathways inside the cell may be used (MyD88 and TRIF pathways).
- Most signals activate **NFKB**, a key regulator of DNA transcription
- End result of intracellular signalling includes innate cell functions:
 - ** production of **cytokines** (chemical messengers)
 - ** Production of **cytolytic** molecules (e.g. in neutrophils)
 - ** production of **interferon** (antiviral substance)
 - ** Activation of **complement** (more on this later)
 - ** Cause **inflammation**
 - ** stimulation of **adaptive immunity** (more on this later)
 - ****increased antigen presentation**

TLR and innate immune response to bacteria



Some pathogens evade innate immunity recognition

e.g. *Streptococcus pneumoniae* bacteria

- causes ear infections, meningitis, pneumonia; Gram positive
- Evasion strategy: has a capsule around cell wall which prevents innate recognition

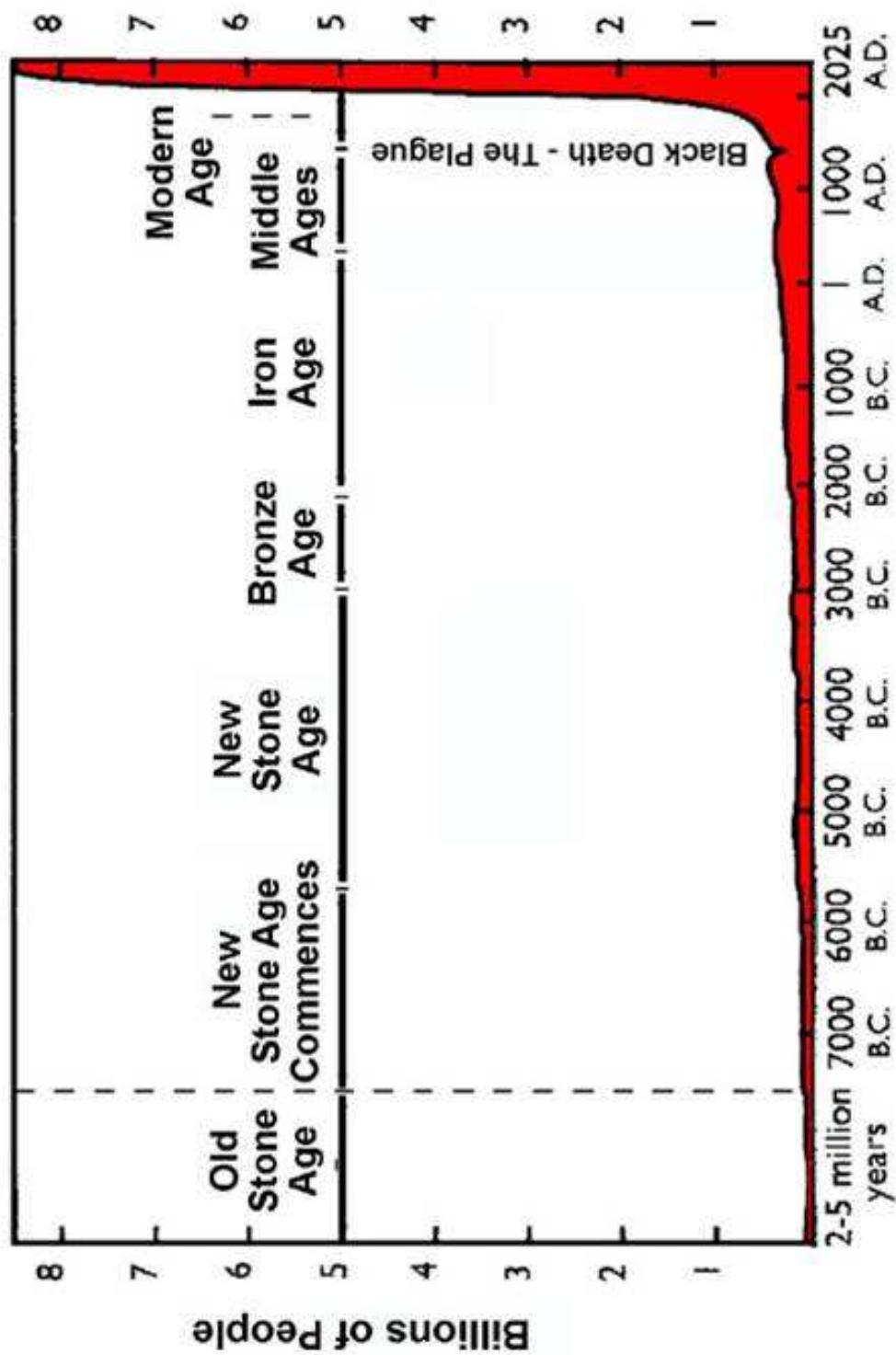
e.g. *Yersinia pestis* bacteria

- causes plague (carried by rat fleas)
- Evasion strategy : a 37 deg C (entry into human host), it starts making a type of LPS that is not recognised by PRR

e.g. *Mycobacterium TB* bacteria

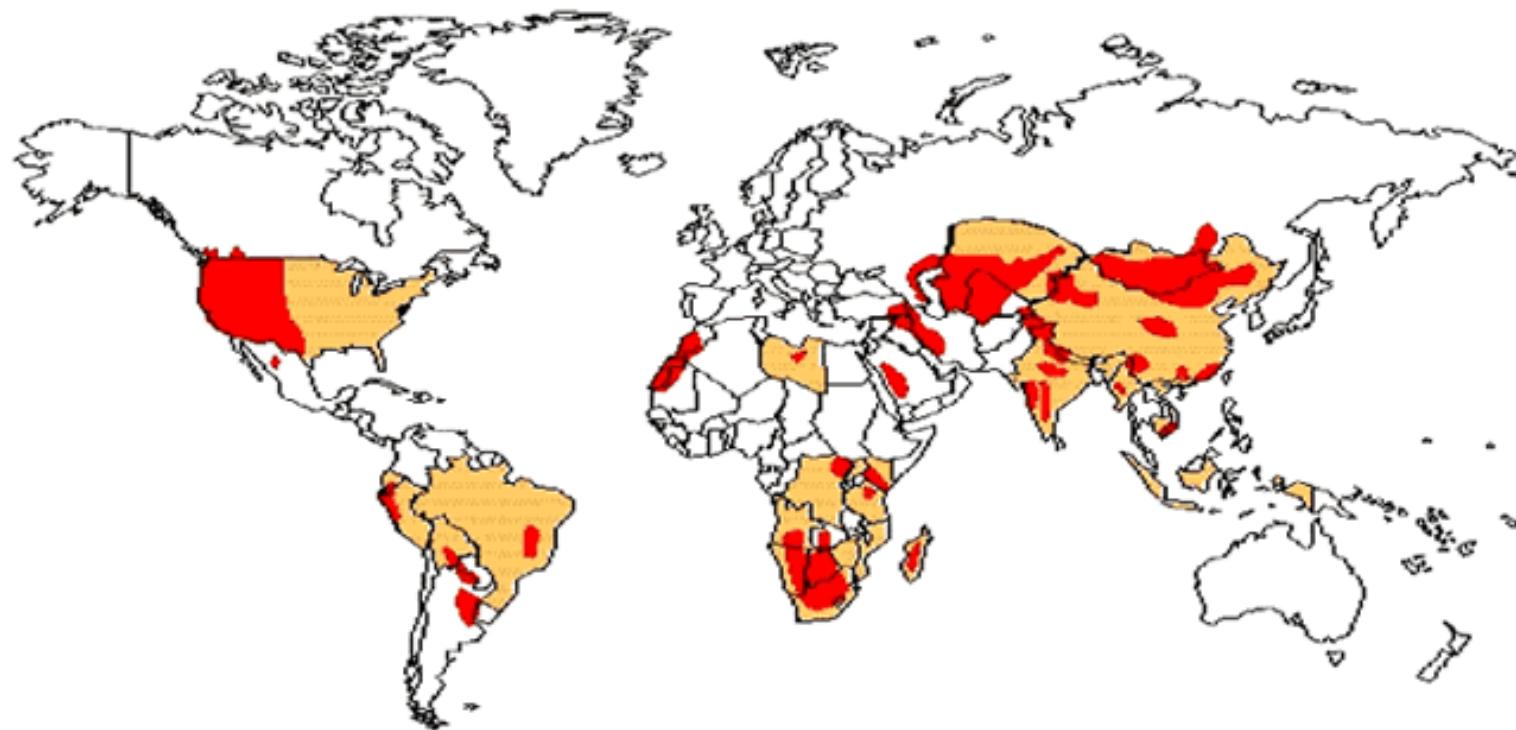
- Causes tuberculosis
- Evasion strategy : after phagocytosis, TB replicates insides macrophages – does not trigger external PRRs

World Population Growth Through History



From "World Population: Toward the Next Century," copyright 1994
by the Population Reference Bureau

World Distribution of Plague, 1998



- Countries reported plague, 1970-1998.
- Regions where plague occurs in animals.

Today: plague is easily treated by antibiotics

Tuberculosis

TB is a curable illness of the respiratory system.



Tuberculosis

- Caused by bacteria that spreads through air and breathed into lungs
- Contagious but curable
- Approx. 2 billion people are infected with TB bacteria (One in 10 infected develops active TB)

Stages

- Infection - Bacteria is alive but body's immune system prevents it from spreading

Person is not sick, not contagious



Pulmonary TB



Mycobacterium tuberculosis

Affected

Normal

Extrapulmonary TB

- Affects the brain, eyes, lymph nodes, throat, spine, bones, skin and kidneys

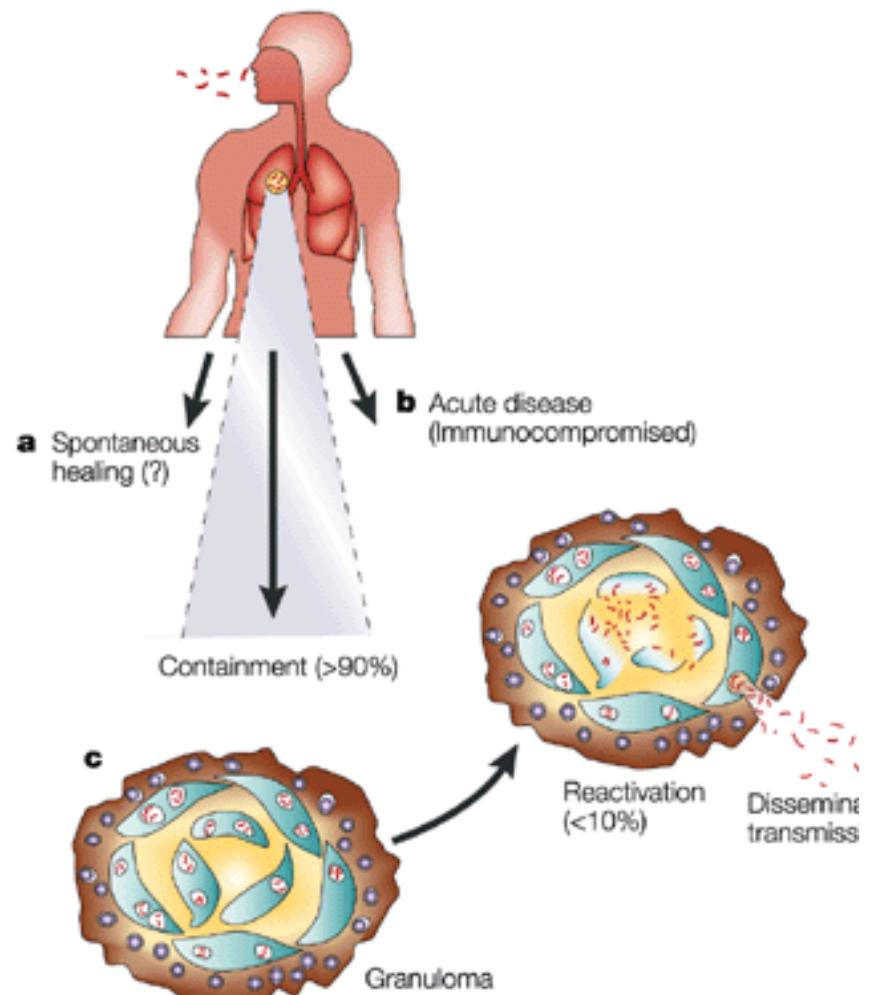


Multi-drug resistant TB

- Strain that thwarts first line of antibiotics used to treat TB
- Caused mainly by misuse of antibiotics, failure to complete full drug treatment

AFR 180402

Source: WHO/CDC

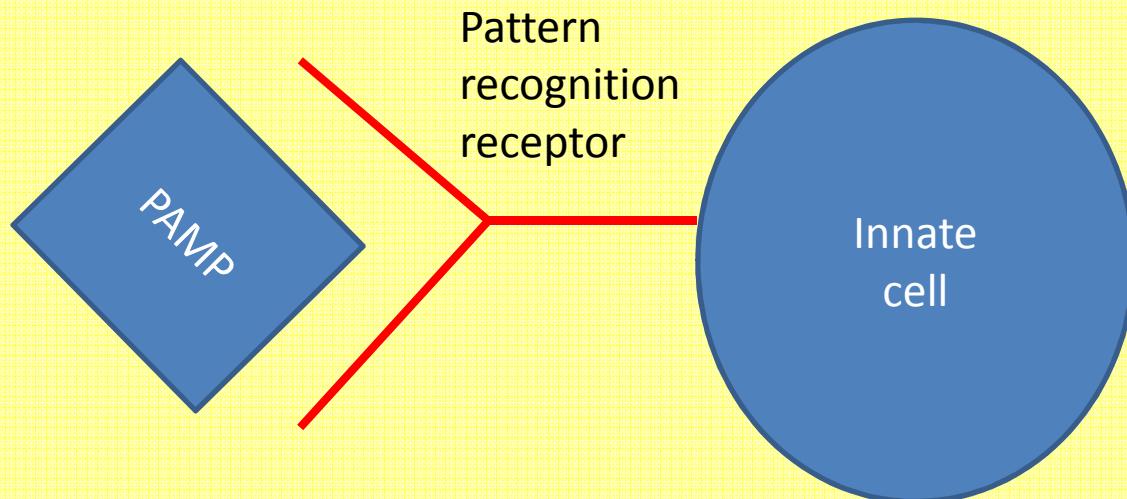


Check your understanding

1. What is a PAMP, what are PRR and how do they interact with each other?
2. Discuss variation in TLR and what they can detect.
3. What is the end result of TLR signalling?
4. what do you think the consequences of mutations in TLR might be?

1. What is a PAMP, what are PRR and how do they interact with each other?

- PAMP= pathogen-associated molecular pattern
- e.g. Components of pathogen cell wall, flagella , DNA or RNA
- PRR= pattern recognition receptor found on innate immune cells e.g. TLR



2. Discuss variation in TLR and what they can detect.

- 10 different types of TLR
- Found on external membrane and internal membranes of innate cells
- Detect PAMPs from pathogens

TLR type	TLR location	PAMP	Source of PAMP
2	surface	lipoteichoic acid; peptidoglycan	Gram positive bacteria
3	Internal	Double stranded RNA	Viruses
4	Surface	Lipopolysaccharide (LPS)	Gram negative Bacteria
5	surface	Flagellin	Bacteria
9	Internal	Unmethylated CpG DNA	Bacteria or viruses

3. What is the result of TLR signalling?

- Intracellular signal transduction via MyD88 or others
- Activate NFKB transcription factor
- Causes DNA transcription
- End result: functions of innate cells
- E.g. cytokine production
- AMP production
- Antigen presentation
- Cytotoxicity
- Etc

consequences of mutations in TLR

- **Mutations that cause increased signalling:**
- = increased cytokine and AMP production, increased antigen presentation, increased cytotoxicity
=more protection from infectious disease
 - e.g. TLR4 180Mal allele protects against malaria and against further infections in HIV+ patients
 - (But the same allele can also increase risk of septic shock ; altered immune function... a delicate balance)

Mutations that cause decreased signalling:

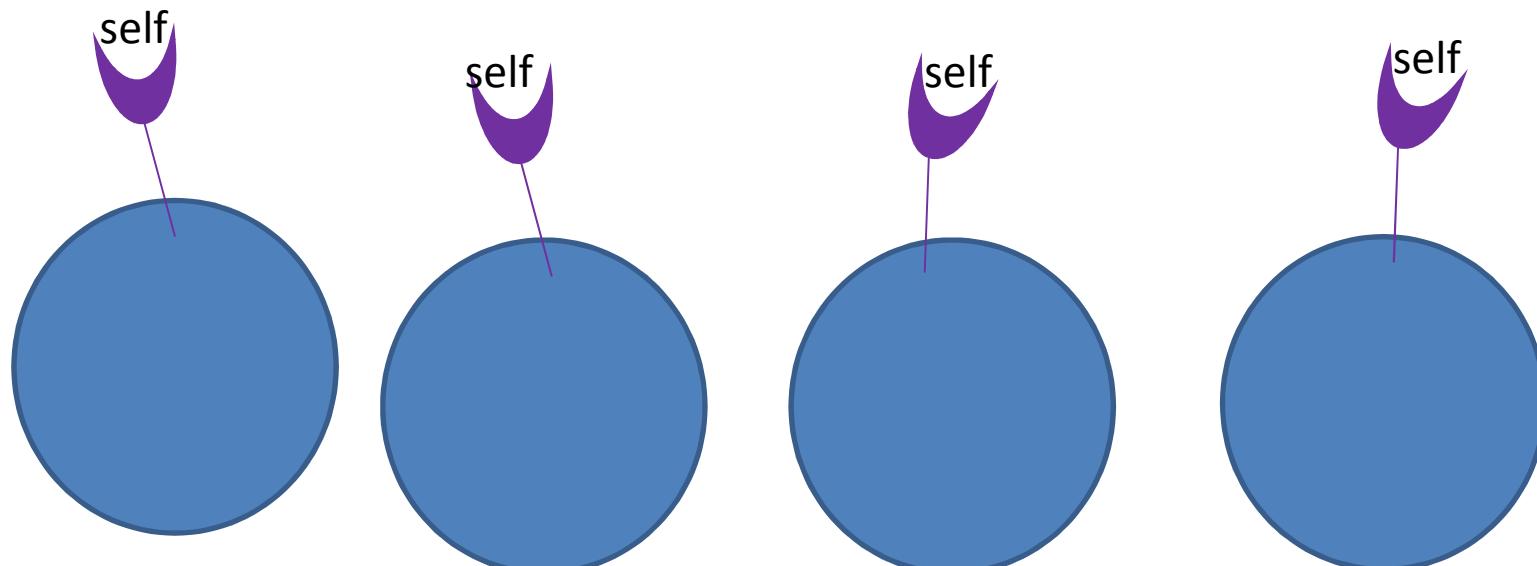
- = decreased cytokine and AMP production, decreased antigen presentation, decreased cytotoxicity
=less protection from infectious disease
 - e.g. TLR4 299GLy cannot respond to LPS, causes increased risk of Gram-negative infections

What receptors are used by NK cells?

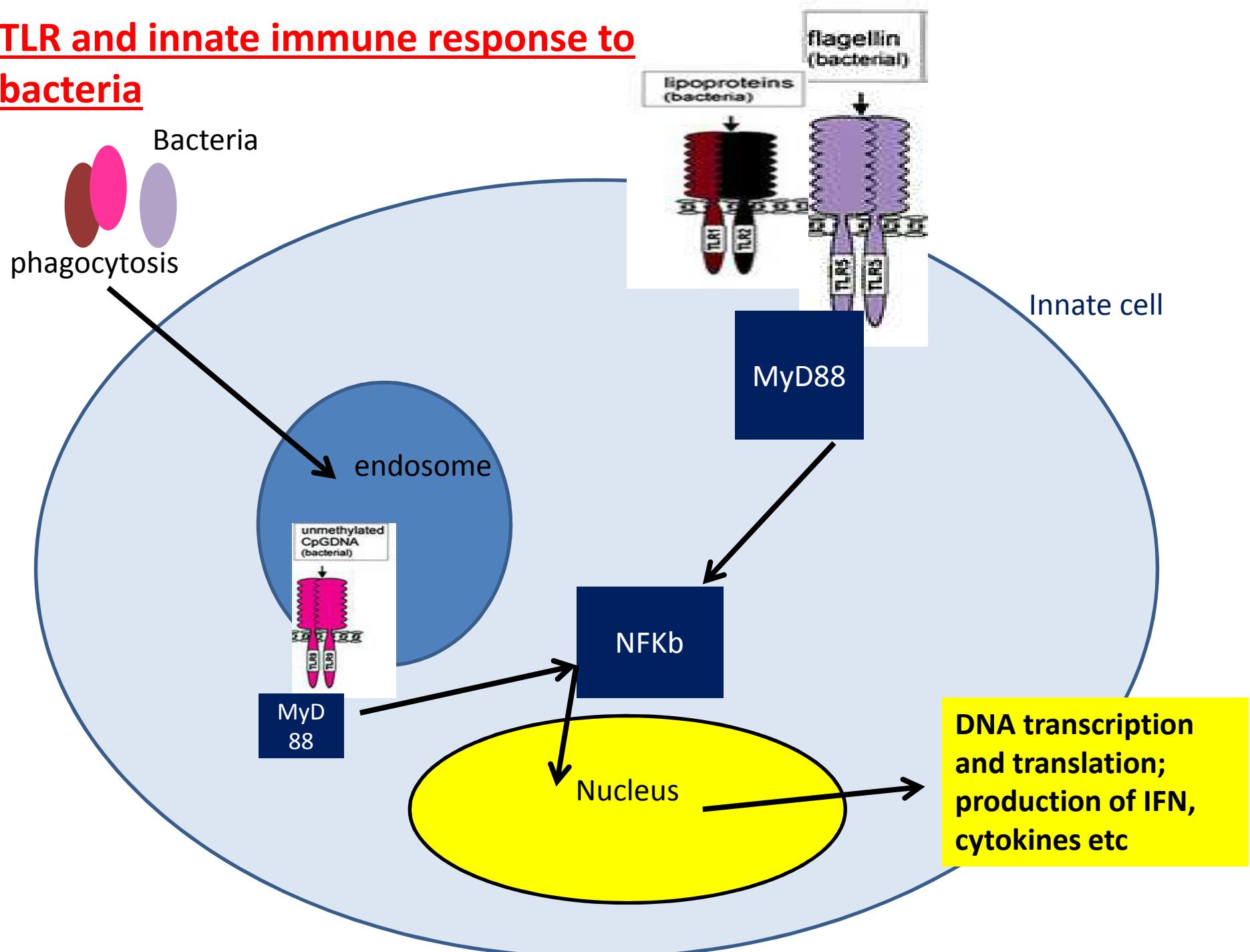
- How do NK recognise pathogens?
- NK cells have a very wide variety of receptors
- One interesting receptor-ligand pair used by NK cells are KIR – HLA

NK cells and HLA

- One of the immune system's jobs is to recognise **self vs. non self**
- **MHC** = major histocompatibility complex;
- also known as **HLA** = human leukocyte antigen
- All the nucleated cells in your body have MHC / HLA molecules on their surface; the MHC hold small peptides that indicate that these cells are “self”

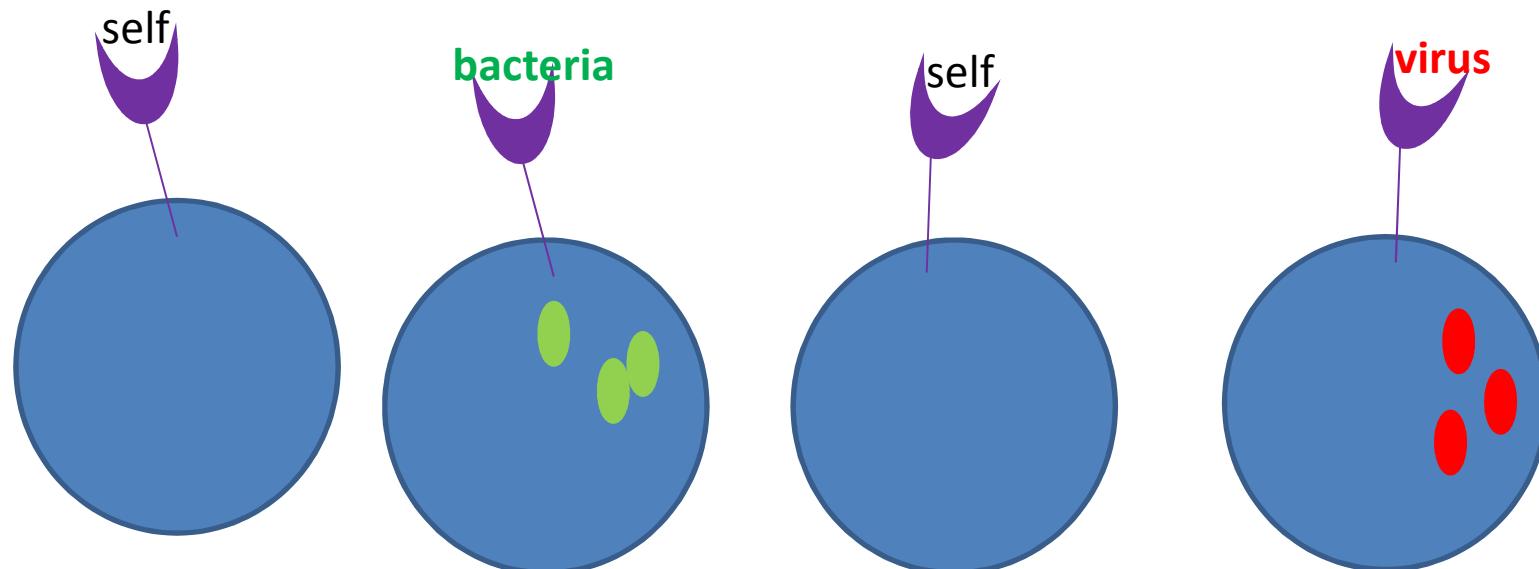


TLR and innate immune response to bacteria



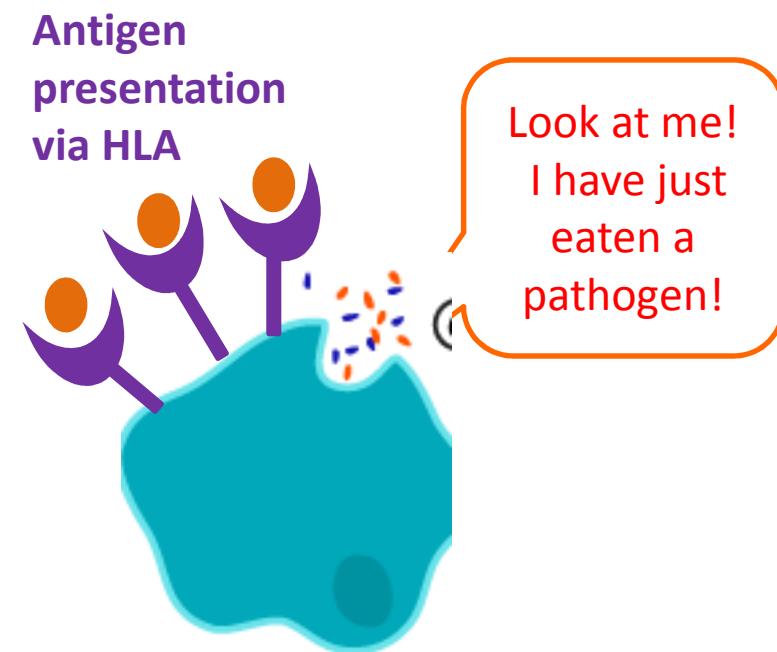
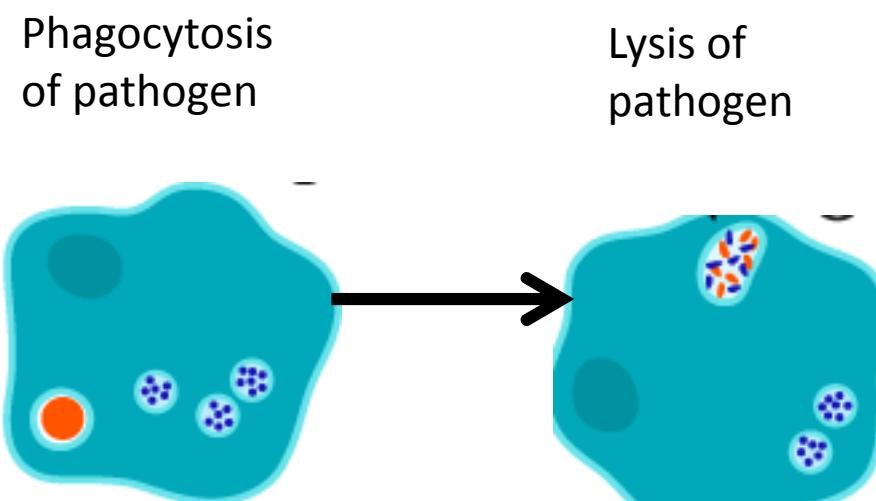
HLA

- MHC (HLA) molecules **can either hold “self” or “not self” peptides**
- The “not self” peptides come from phagocytosis of pathogens
- This is known as antigen presentation



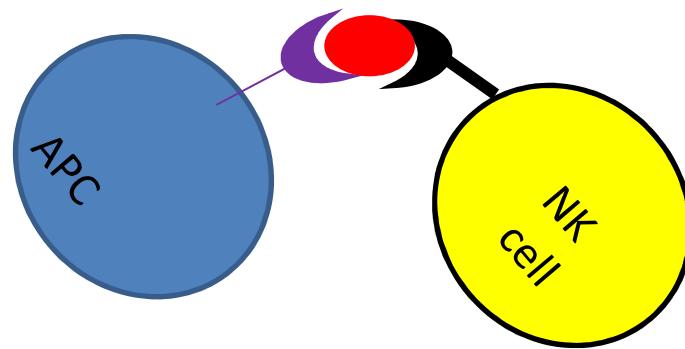
Antigen presentation

- After phagocytosis, the phagocyte keeps some small pieces of the pathogen known as antigens
- The antigens are displayed on the cell surface of the phagocyte
- This is a signal to show that pathogens have been detected
- This only occurs in certain phagocyte cell types known as antigen presenting cells, such as macrophages or dendritic cells
- Uses MHC / HLA



NK cells (natural killer cells)

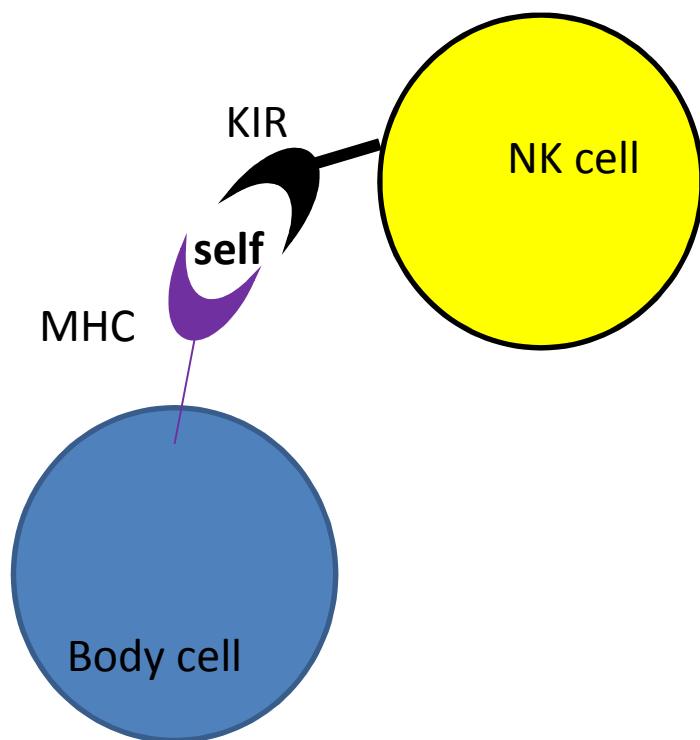
- NK cells have receptors for HLA+presented peptide
- These receptors are called **KIR** (killer cell immunoglobulin-like receptors).
- **NK- KIR and cell-HLA interact;**
- When 2 immune cells physically contact each other, this is called **an immune synapse**



- the NK cell checks to see if the cell is “self” or “not self”

NK-KIR check 3 things:

1. The right HLA is present
2. The amount of HLA
3. If the peptide held by HLA is self or not self



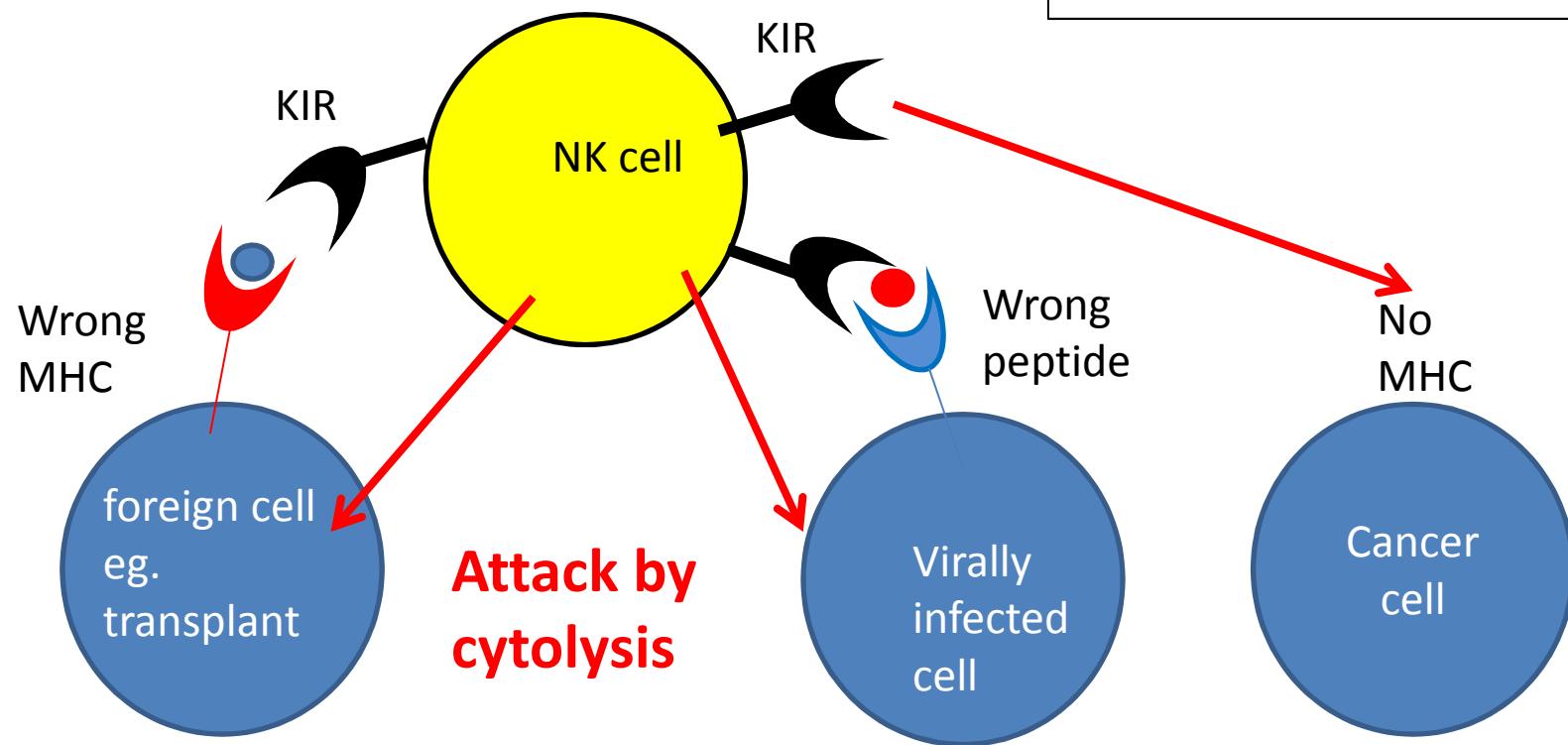
if the NK KIR detects
the right HLA+ self
peptide, it does not
attack the cell

NK-KIR check 3 things:

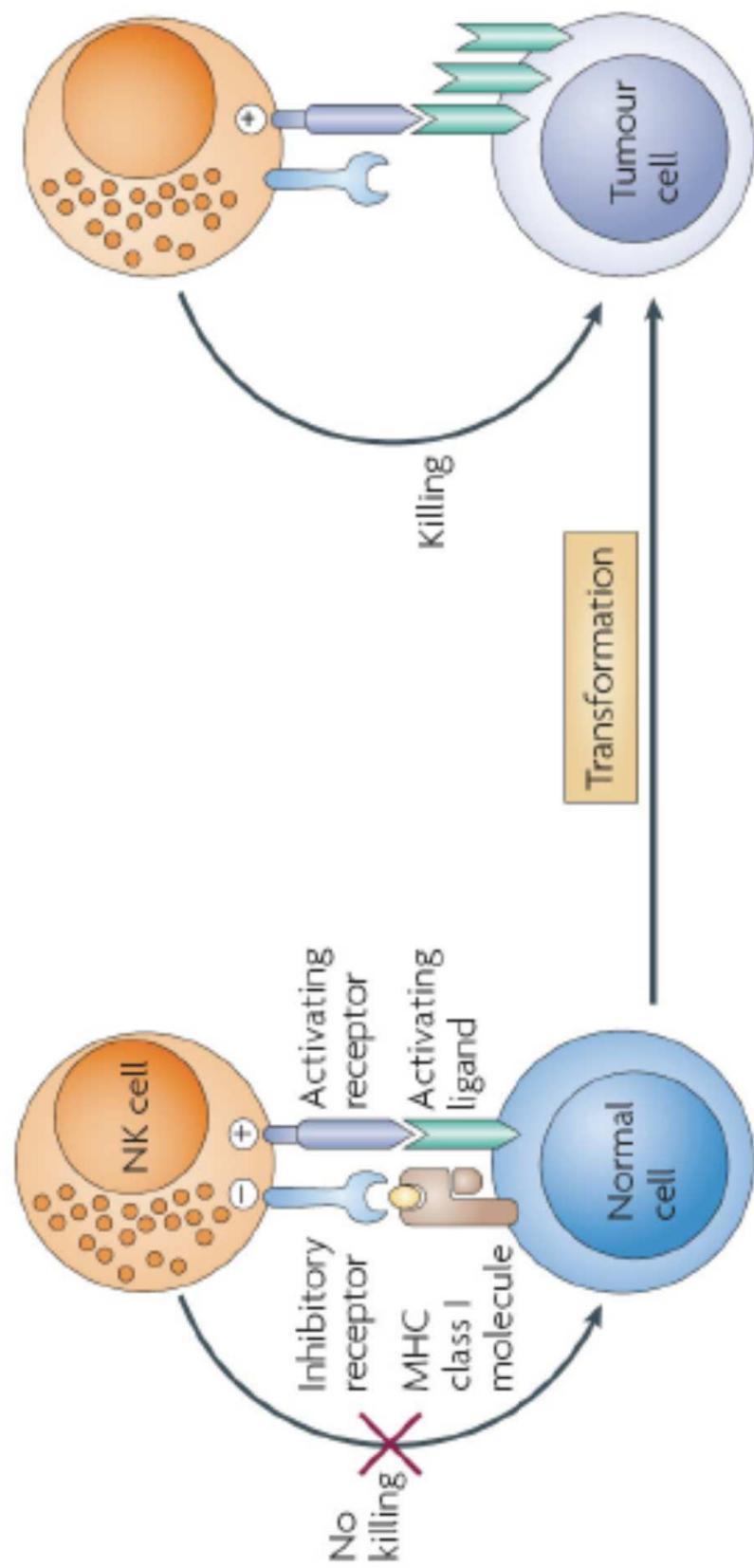
1. The right HLA is present
2. The amount of HLA
3. If peptide held by HLA is self or not self

if the NK KIR detects
NO HLA or the WRONG HLA
NO or LOW HLA
NON-SELF PEPTIDE

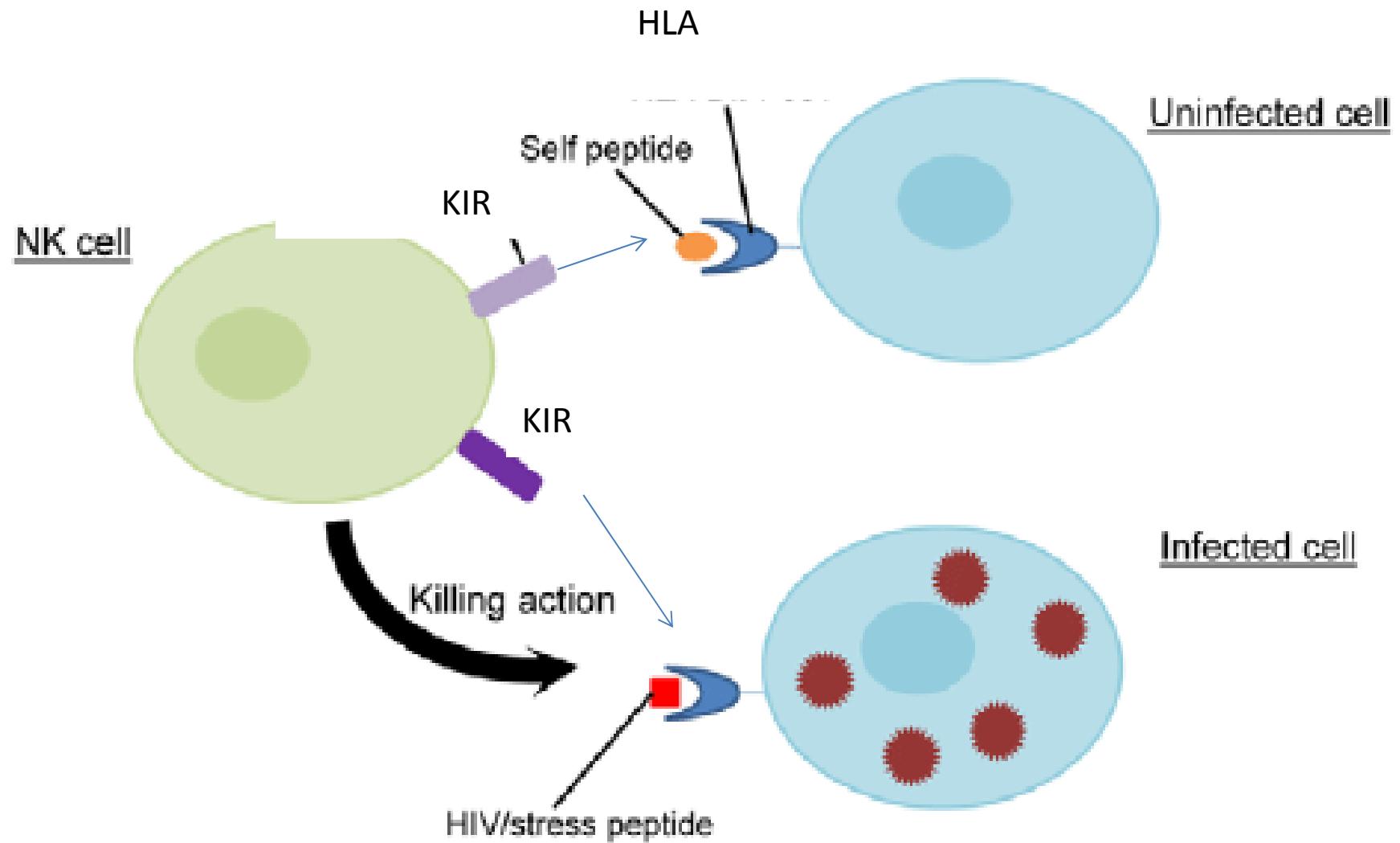
the NK cell attacks the other cell by cytotoxicity
(lyses the other cell)



The missing self hypothesis



Ljunggren and Malmberg, *Nature Reviews Immunology*, May 2007



Tissue or organ transplants

- HLA is also called **MHC=major histocompatibility complex**
- i.e. determines what is compatible for tissue transplants
- HLA are the **most variable genes/ proteins** in the body
- Nearly everyone has different types of HLA
- For a organ transplant, you need to find some one who has the **same HLA type as you (a match)**
- Not easy to find a match
- **If you get an organ/tissue with the wrong type of HLA (not matching), your NK cells attack the donated tissue and try to destroy it**

Why is HLA so diverse?

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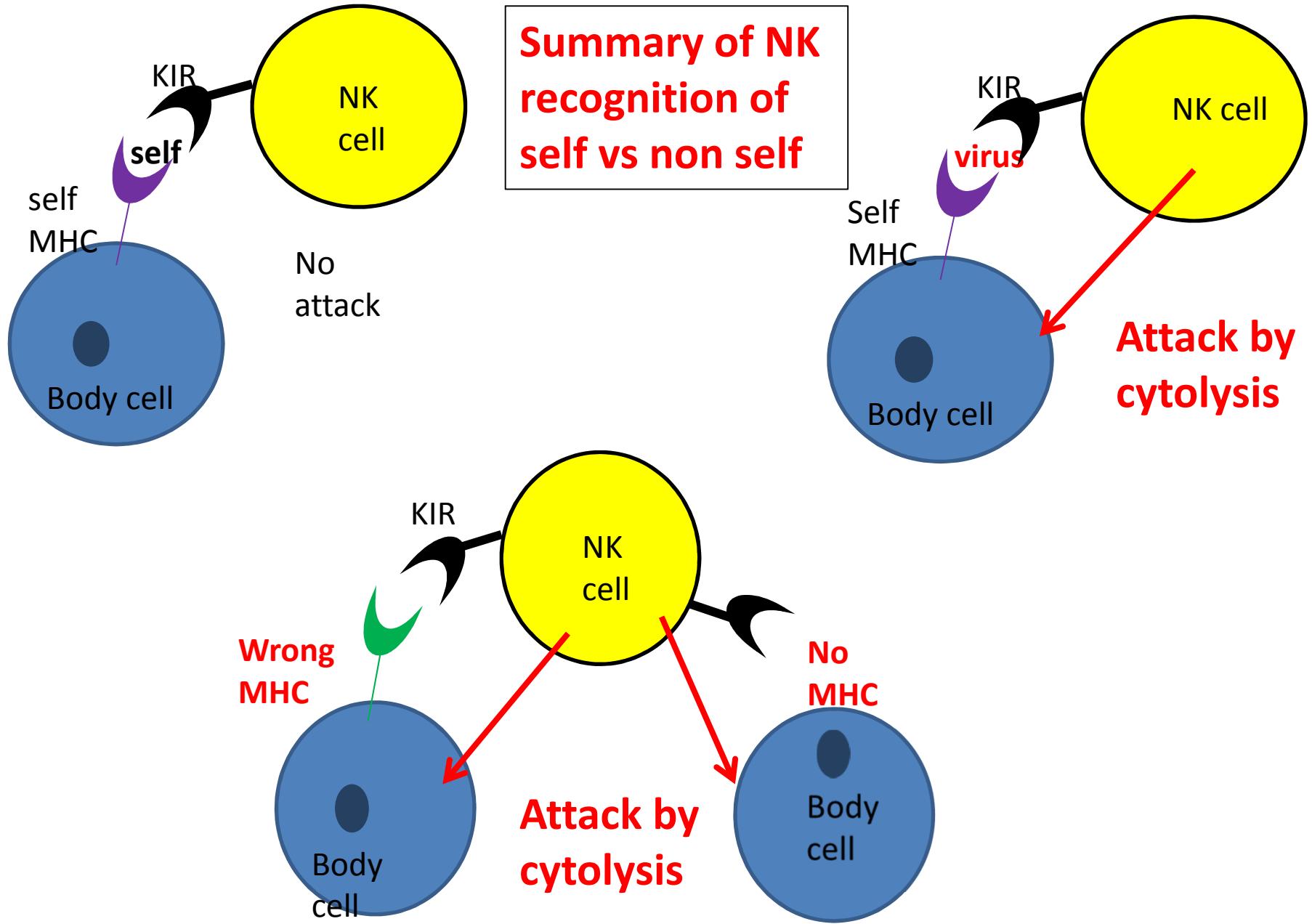
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OUR EVENTS

STORIES

MEDIA

- join the South African Bone Marrow Registry
- Register to become a potential donor
- need blood stem cells for people with cancer or other blood problems
- You will only be asked to donate if they find a match
- Nearly same process as regular blood donation
- The approx probability of finding a match within your own ethnic group is **1:100 000**,
- which means in South Africa we need to recruit 100 000 of each of the four prominent ethnic groups – Black, Coloured, Indian and White.
- =**need 400 000 people**
- **Current: 71 000 people**



Innate immunity and inflammation

- the classical signs of acute inflammation are : heat, pain, redness and swelling
- Response to injury and infection
- Can occur internally and externally
- Can be short-term (acute) or long term (chronic)



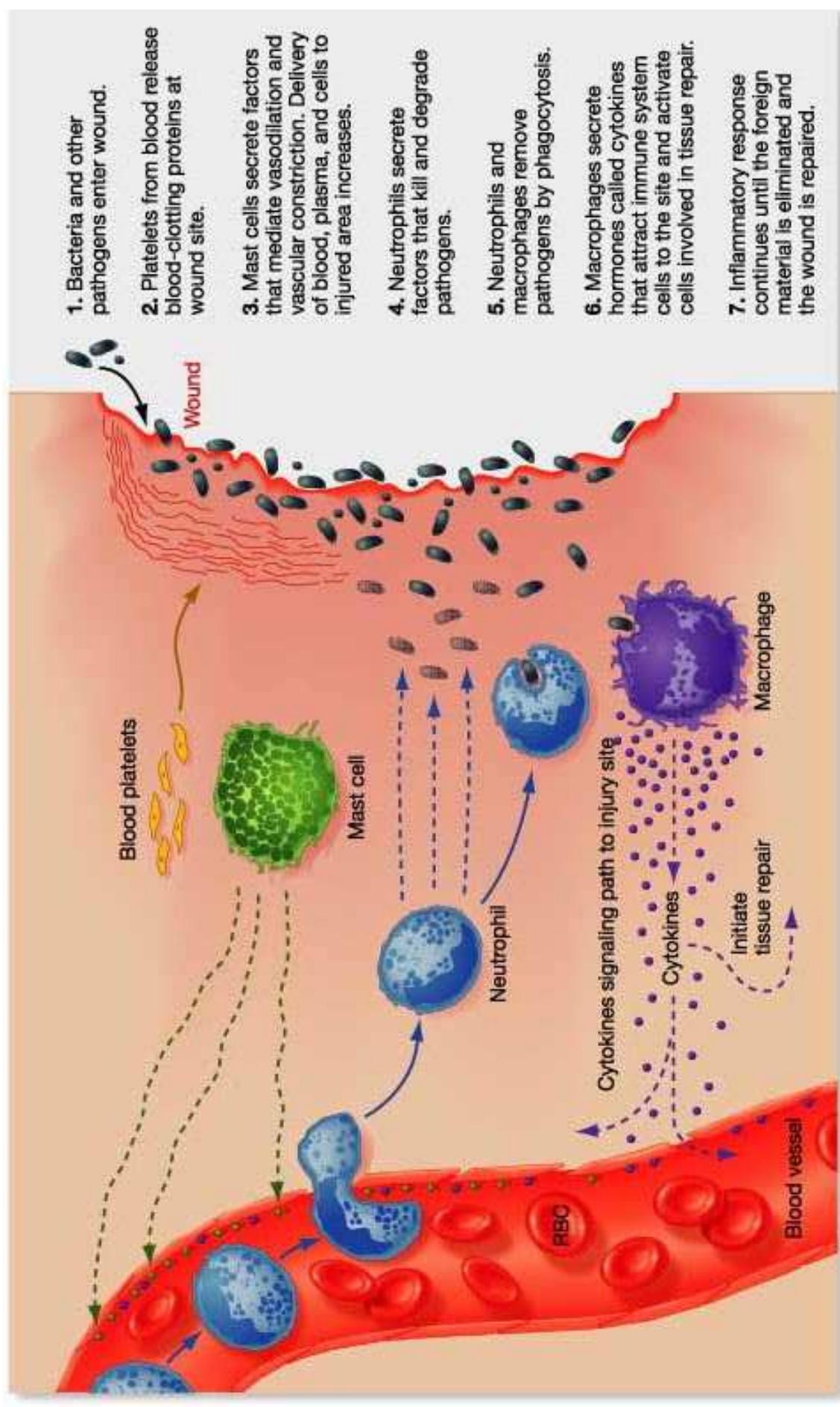
- How is this an immune response?

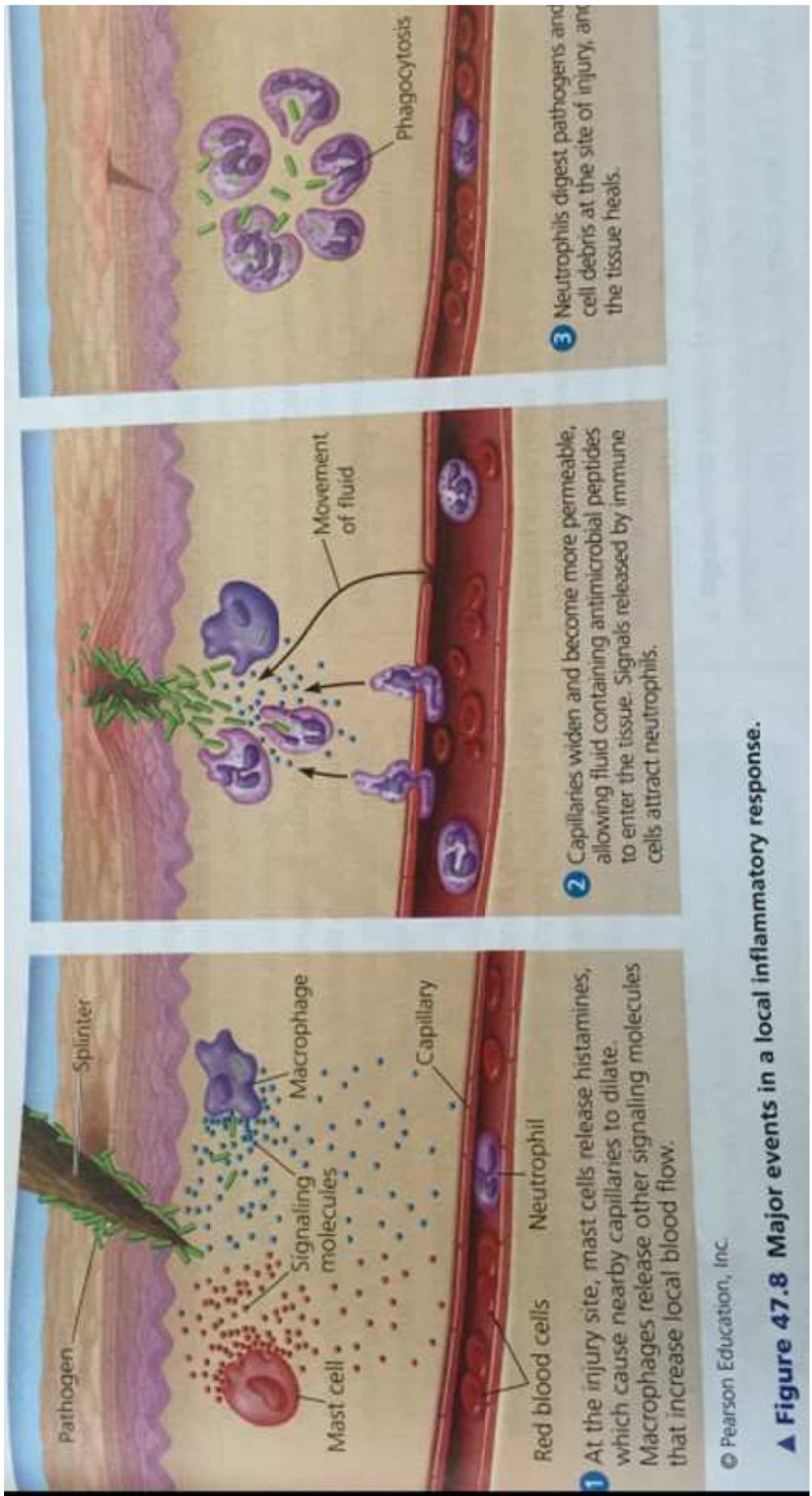
Innate immunity and inflammation

- The function of inflammation is to
 - eliminate the initial cause of cell injury / infection
 - clear out necrotic cells and damaged tissues
 - initiate tissue repair
- Inflammation is a generic (non-specific) response, and therefore it is considered as a mechanism of innate immunity
- Acute inflammation can be summarised as a local response which attracts innate cells out of the blood into affected tissue so that they can respond to pathogens and tissue damage .

Typical steps in acute inflammation

1. Injury or infection occurs (entry of pathogen into host)
2. tissue innate cells like mast cells and macrophages
 - 2a. PRRs recognise PAMPs and are activated
 - 2b. release inflammatory chemicals such as histamine
3. Histamine makes local blood vessels become more permeable
 - 3a. they leak fluid into neighbouring tissue causing swelling
 - 3b. They allow blood innate cells and platelets to migrate out into tissue (extravasion)
4. Innate cells in blood like macrophages and neutrophils
 - 4a. migrate out of the blood vessels into neighbouring tissue – perform phagocytosis
 - 4b. release cytokines which promote blood flow to the area (increased redness) and promote tissue repair
5. Pus may develop = dead pathogens, white blood cells and debris from damaged cells





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▲ **Figure 47.8 Major events in a local inflammatory response.**

Check your understanding

1. Why is inflammation considered an innate immune response and what is the role of innate cells in acute inflammation?