INNATE IMMUNITY

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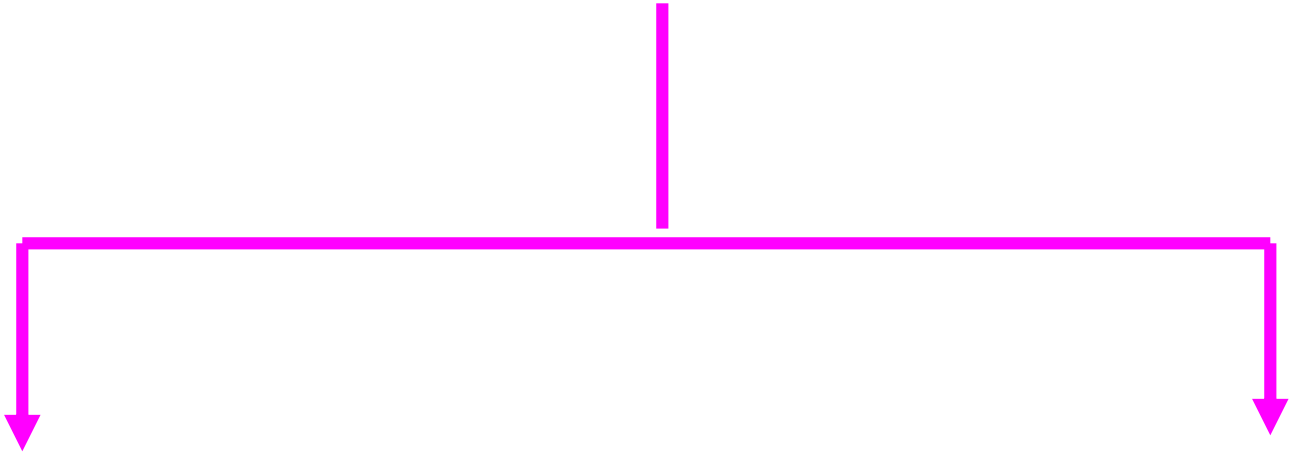
Innate Immunity - characteristics

- Most primitive type of immune system found in virtually all multicellular animals
- high discrimination of host and pathogen
- First line of defense against infection
- no need for prolonged induction
- act quickly
  - immediate direct response 0-4 hrs
  - rapid induced 4-96 hrs
- antigen-independent

Innate Immunity – characteristics (contd.)

- dependence on germ line encoded receptors
- always present and active, constitutively expressed
  (some components can be up-regulated)
- Nonspecific; not specifically directed against any particular infectious agent or tumor
- no clonal expansion of Ag specificity
- Same every time; no ‘memory’ as found in the adaptive immune system
- failure ==> adaptive immune response

Components of Innate Immunity



First line

1 Physical barriers

2 Chemical & biochemical barriers

3 Biological barriers (Normal flora)

Second line

A- cells

1- Natural killer

2- Phagocytes

3- inflammatory cells

B- Soluble factors

C- Inflammatory barriers

Anatomical /Physical/Mechanical Barriers

System or Organ	Cell type	Mechanism
Skin	Squamous epithelium	Physical barrier (intact skin) Desquamation
Mucous Membranes	Non-ciliated epithelium (*e.g.* GI tract)	Peristalsis
	Ciliated epithelium, hairs (*e.g.* respiratory tract)	Mucociliary elevator, Coughing, sneezing
	Epithelium (*e.g.* nasopharynx)	Flushing action of tears, saliva, mucus, urine; blinking of eye lids

Biological Factors

System or Organ	Component	Mechanism
Skin and mucous membranes	Normal microflora	Antimicrobial substances Competition for nutrients and colonization

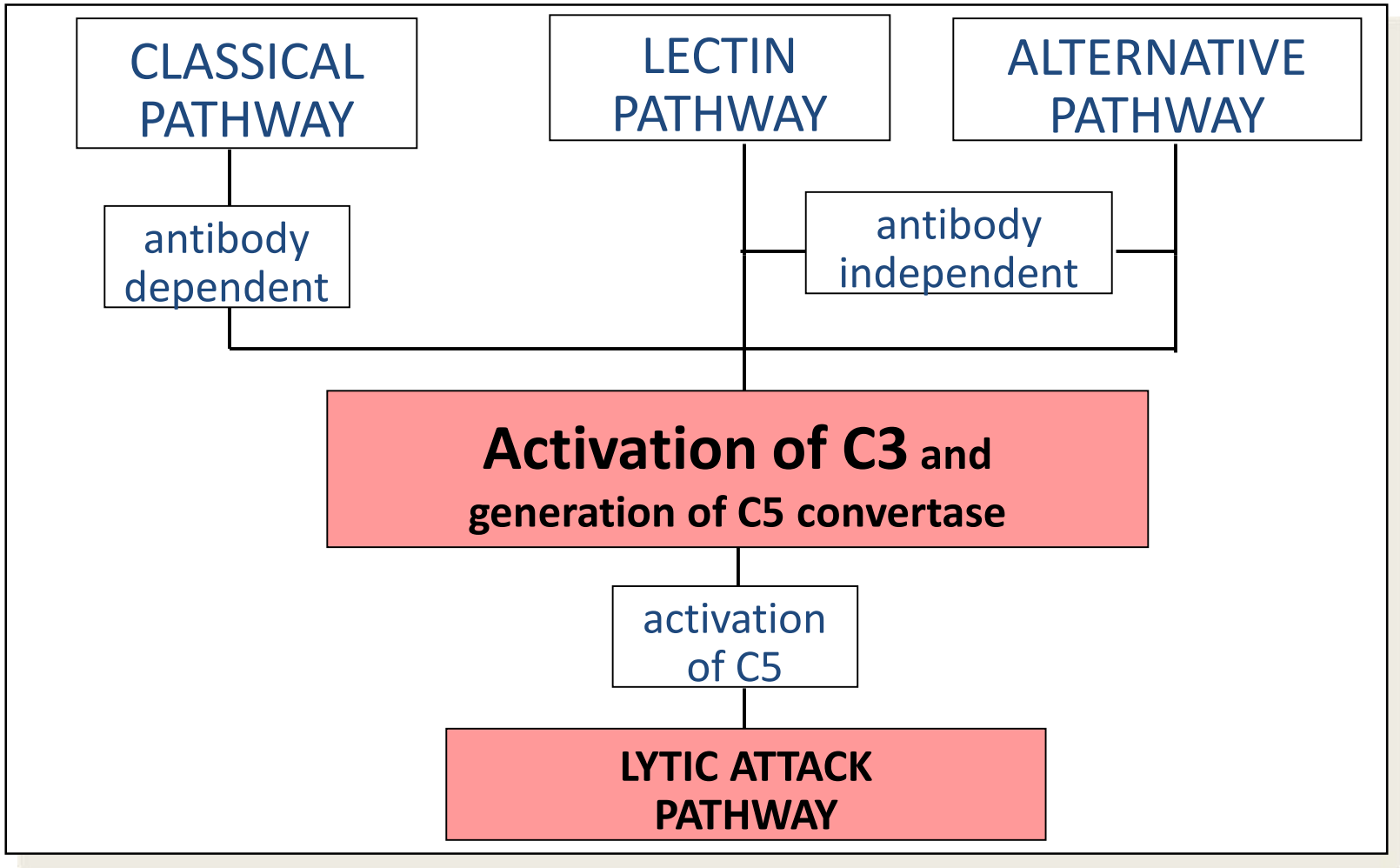
Chemical Factors
(at surfaces and in body cavities)

System or Organ	Component	Mechanism
Skin	Sweat	Anti-microbial fatty acids, high salt conc.
Mucous Membranes	HCl (parietal cells) Tears and saliva	Low pH Lysozyme and phospholipase A
	Defensins (respiratory & GI tract)	Antimicrobial
	Sufactants (lung)	Opsonin

Chemical Factors
(Humoral Components)

Component	Mechanism
Complement	Lysis of bacteria and some viruses, Opsonin Increase in vascular permeability Recruitment and activation of phagocytic cells
Coagulation system	Increase vascular permeability, Recruitment of phagocytic cells, β-lysin from platelets – a cationic detergent, antibacterial
Acute phase protein	Antibacterial
Lysozyme	Breaks down bacterial cell walls
Lactoferrin and transferrin	Compete with bacteria for iron
Cytokines	Various effects
Interferon	Anti-viral protein

Pathways of complement activation



THE ACUTE PHASE PROTEINS

- 'Acute phase proteins' are a large group of plasma proteins whose concentration increases (or decreases) (by 25% or more) during inflammation (acute or chronic), such as injury and in disease states.
- Proinflammatory cytokines stimulate hepatocytes in the liver to synthesize and secrete acute phase proteins.
- Examples of acute phase proteins are CRP, MBP, haptoglobin, SAA, fibrinogen, α_1-antitrypsin, and complement components C3 and C4.
- C-reactive protein (CRP) binds to membrane phospholipids in microbial membranes.
- Mannose binding protein (MBP) binds to mannose sugars found in many bacteria and fungi.
- These functions as opsonins, soluble pattern-recognition receptors, activate the complement pathway or be involved in sequestration of essential nutrients.

Antimicrobial Peptides (AMPs)

- **Antimicrobial peptides** forms pores in the cytoplasmic membrane of a variety of bacteria causing leakage of cellular needs, e.g. lysozymes, lactoferrins, defensins, protegrins, granulozymes, etc

- **Lysozyme**, in serum, mucus, plasma, tissue fluids and tears, breaks down the bacterial cell wall (peptidoglycan)

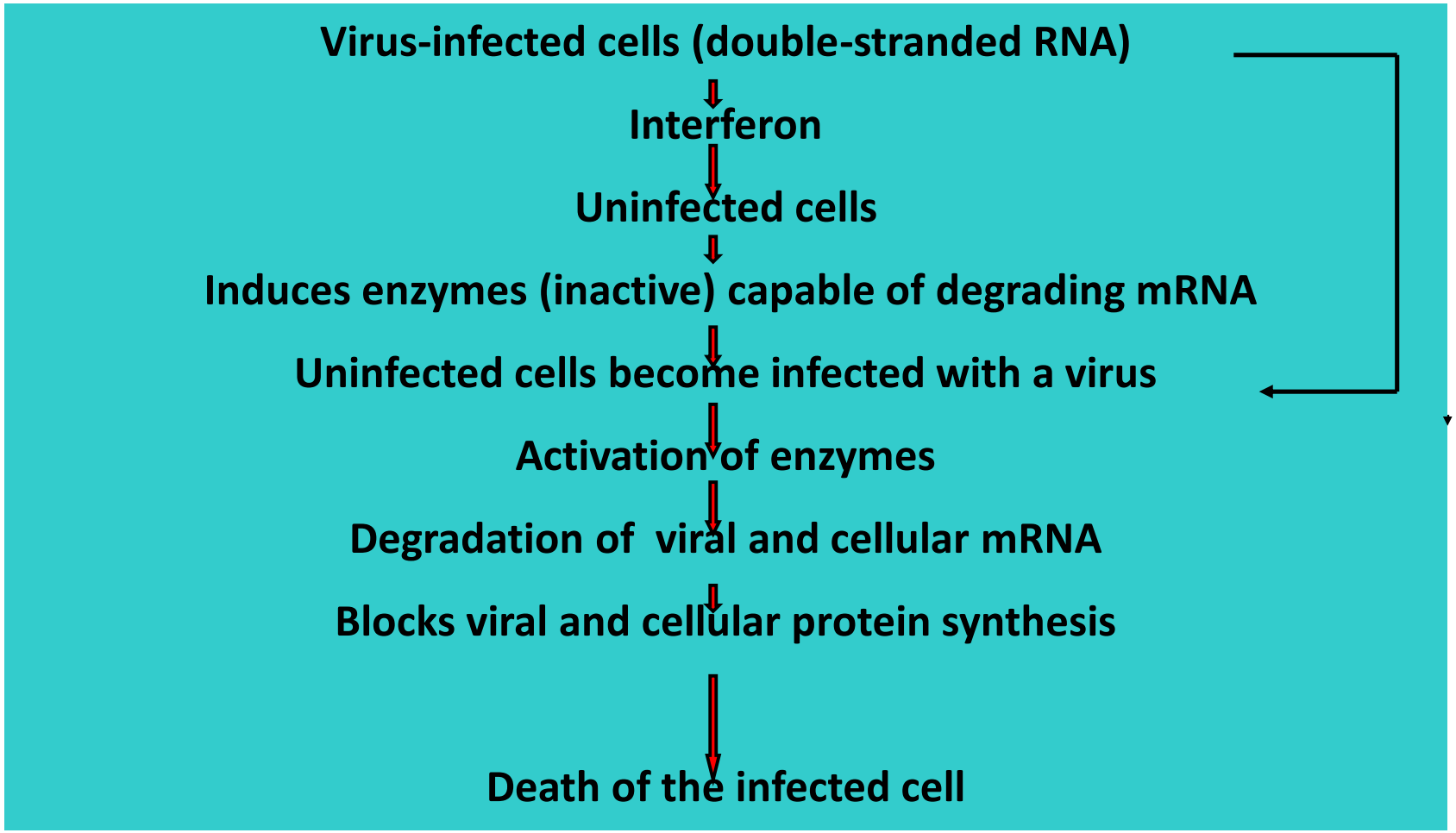
- **Beta-defensins** are short peptides found in blood plasma and mucous.

- **Transferrin & lactoferrin** competitively binds iron in blood, tissues and milk thereby preventing its availability to microorganisms

Interferons (IFN)

- Interferons (IFNs) comprise a family of secreted α-helical cytokines induced in response to specific extracellular biomolecules of viruses or other pathogens through stimulation of Toll-like receptors (TLRs).
- Act in paracrine or autocrine modes for regulating innate and acquired immunity, resistance to viral infections, and normal and tumor cell survival and death.
- There are five types of human interferon: **alpha, beta, gamma, delta and omega (α -IFN**, **β -IFN**, **γ-IFN**, **ξ-IFN**, and **ω-IFN**, respectively) .
- **Virus** infected cells produce **IFN-α and IFN-β**.
- Interferons are **host-cell-specific, but not virus-specific**
- **Gamma-IFN**, also called as immune interferon, activates neutrophils, NK cells and macrophages.
- Interferons also result in
  resistance to viral replication; induce enzymes to degrade viral mRNA
  increased MHC I expression
  activate NK cells, T-cells and macrophages

Interferons



Cellular Components

Cell	Functions
Neutrophils	Phagocytosis and intracellular killing Inflammation and tissue damage
Macrophages	Phagocytosis and intracellular killing Extracellular killing of infected or altered self targets Tissue repair
NK cells	Killing of virus-infected and altered self targets
Eosinophils	Killing of certain parasites

Inflammation

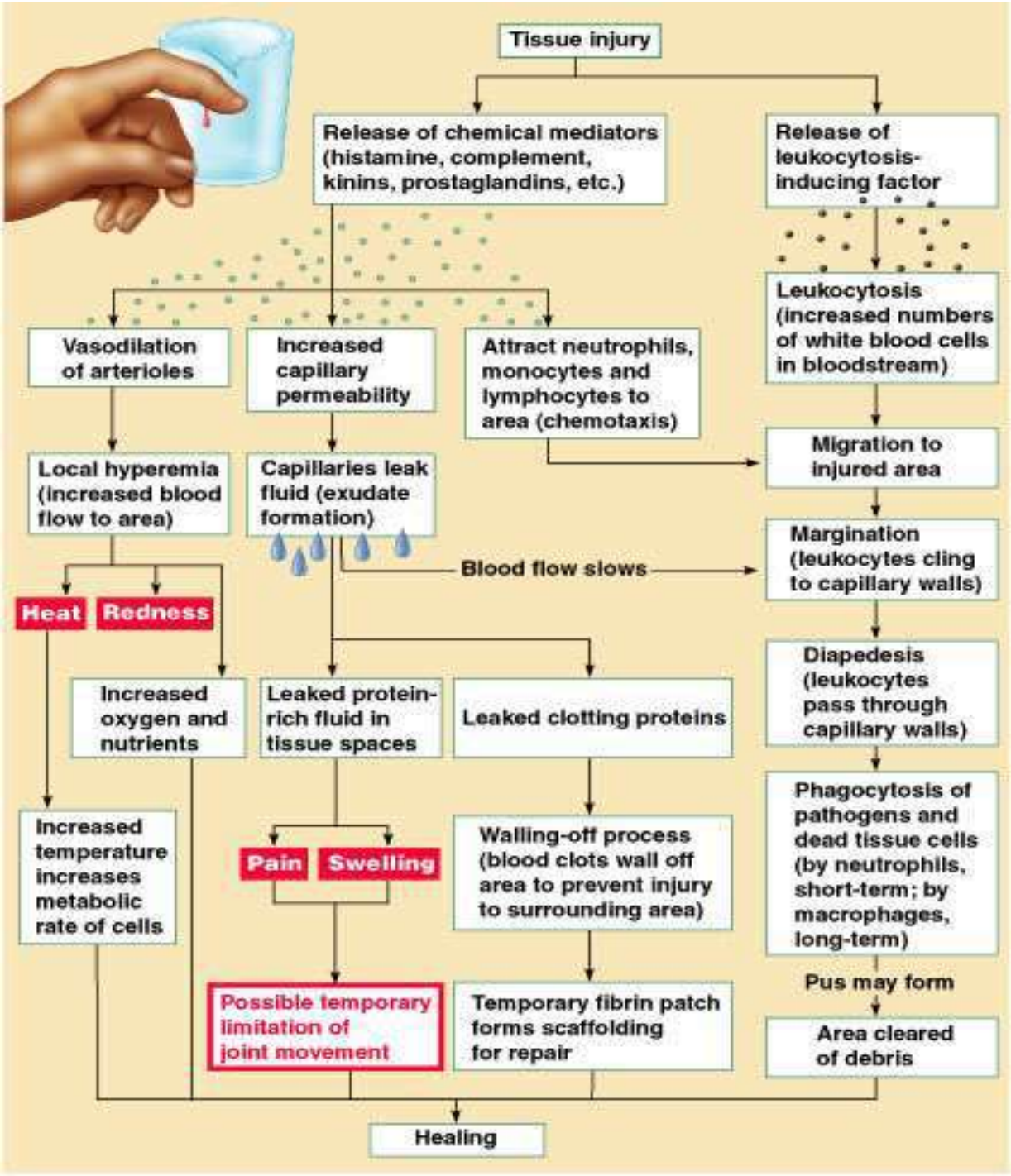
Inflammation

- Inflammation is an attempt by the body to eliminate the noxious agent and restore and maintain homeostasis after injury to a tissue.
- The injury is often caused by invading organisms.
- It is the second line of defense.

Inflammation (contd.)

The principal effects of inflammation to a site of injury are:

- An increase in blood supply.
- An increase in vascular permeability to large serum molecules.
- Enhanced migration of leukocytes across the local vascular endothelium and in the direction of the site of inflammation.
- The inflammatory response is characterized by redness, heat, swelling and pain.


Tissue injury
Release of chemical mediators (histamine, complement, kinins, prostaglandins, etc.)
Release of leukocytosis-inducing factor
Vasodilation of arterioles
Increased capillary permeability
Attract neutrophils, monocytes and lymphocytes to area (chemotaxis)
Leukocytosis (increased numbers of white blood cells in bloodstream)
Local hyperemia (increased blood flow to area)
Capillaries leak fluid (exudate formation)
Migration to injured area
Blood flow slows
Margination (leukocytes cling to capillary walls)
Heat
Redness
Increased oxygen and nutrients
Leaked protein-rich fluid in tissue spaces
Leaked clotting proteins
Diapedesis (leukocytes pass through capillary walls)
Increased temperature increases metabolic rate of cells
Pain
Swelling
Walling-off process (blood clots wall off area to prevent injury to surrounding area)
Phagocytosis of pathogens and dead tissue cells (by neutrophils, short-term; by macrophages, long-term)
Pus may form
Possible temporary limitation of joint movement
Temporary fibrin patch forms scaffolding for repair
Area cleared of debris
Healing

The Good Side of Inflammation

The inflammatory response to tissue damage is of great value by:

- isolating the damaged area
- mobilizing effector cells and molecules to the site, and
- in the late stages — promoting healing

Inflammation protects the body (innate immunity)

The Bad Side of Inflammation

Often the inflammatory response is out of proportion to the threat it is dealing with. The result can be more damaging to the body than the agent itself would have produced.

Allergies and Autoimmune Diseases are examples of inflammation in response to what should have been a harmless, or at least noninfectious, agent

Phagocytosis

Phagocytosis

- **Phagocytosis** is the ingestion of microorganisms or particulate matter by a cell.
- Phagocytosis is performed by **phagocytes**—certain types **of white blood cells** or derivatives of them.

All phagocytes
eat, digest
and extrude

Phagocytes and Their Relatives

Monocyte

Eosinophil

Mast cell

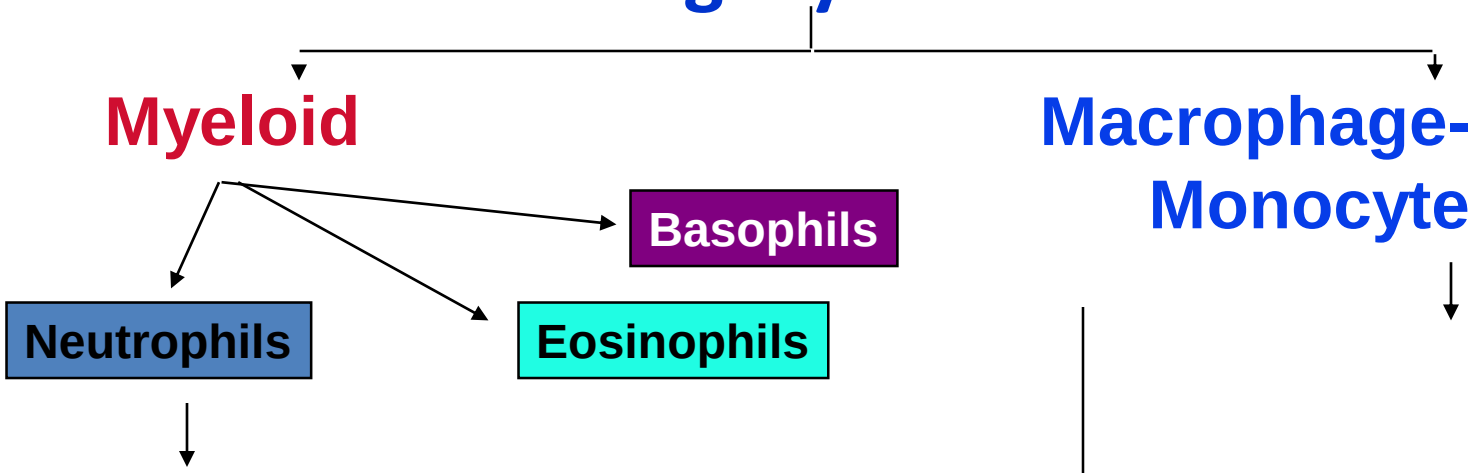
Macrophage

Dendritic cell

Neutrophil

Basophil

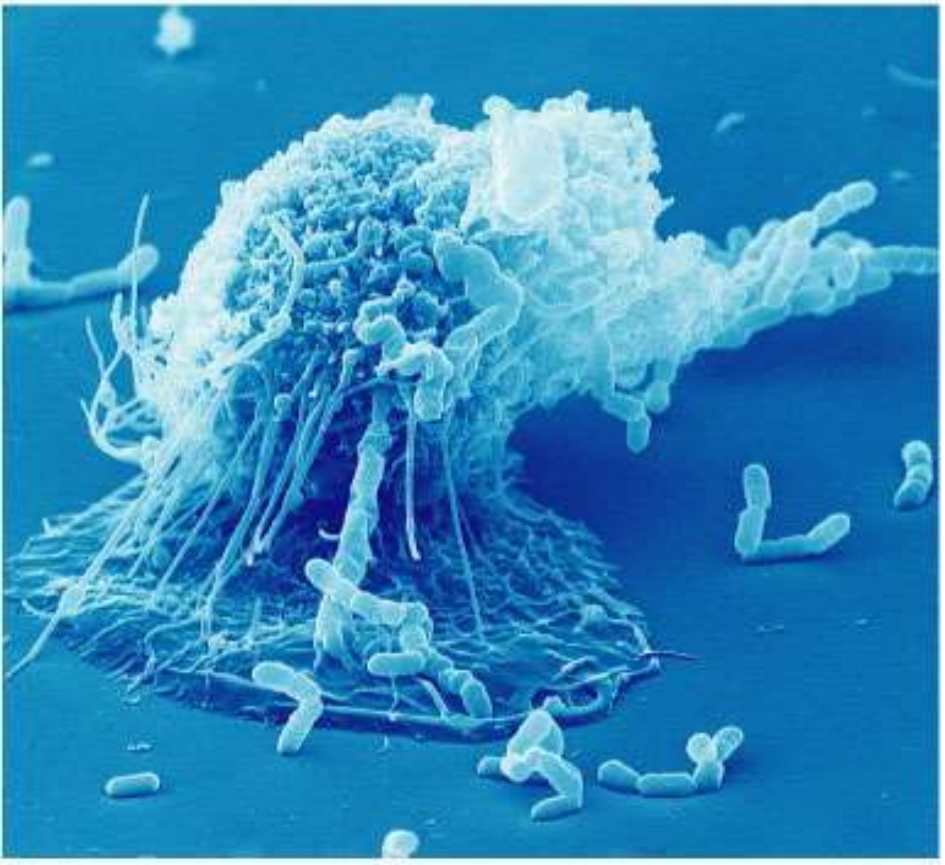
Phagocytic Cells



- Rapid phagocytosis, but cannot phagocytose repeatedly
- Has granules which contain bactericidal enzymes
- Short lived
- NO ABILITY TO PRESENT ANTIGEN

- Slow but can phagocytose repeatedly.
- Contain bactericidal enzyme.
- Long lived
- Selected cells HAVE ability to present Ag.

Mechanism of Phagocytosis



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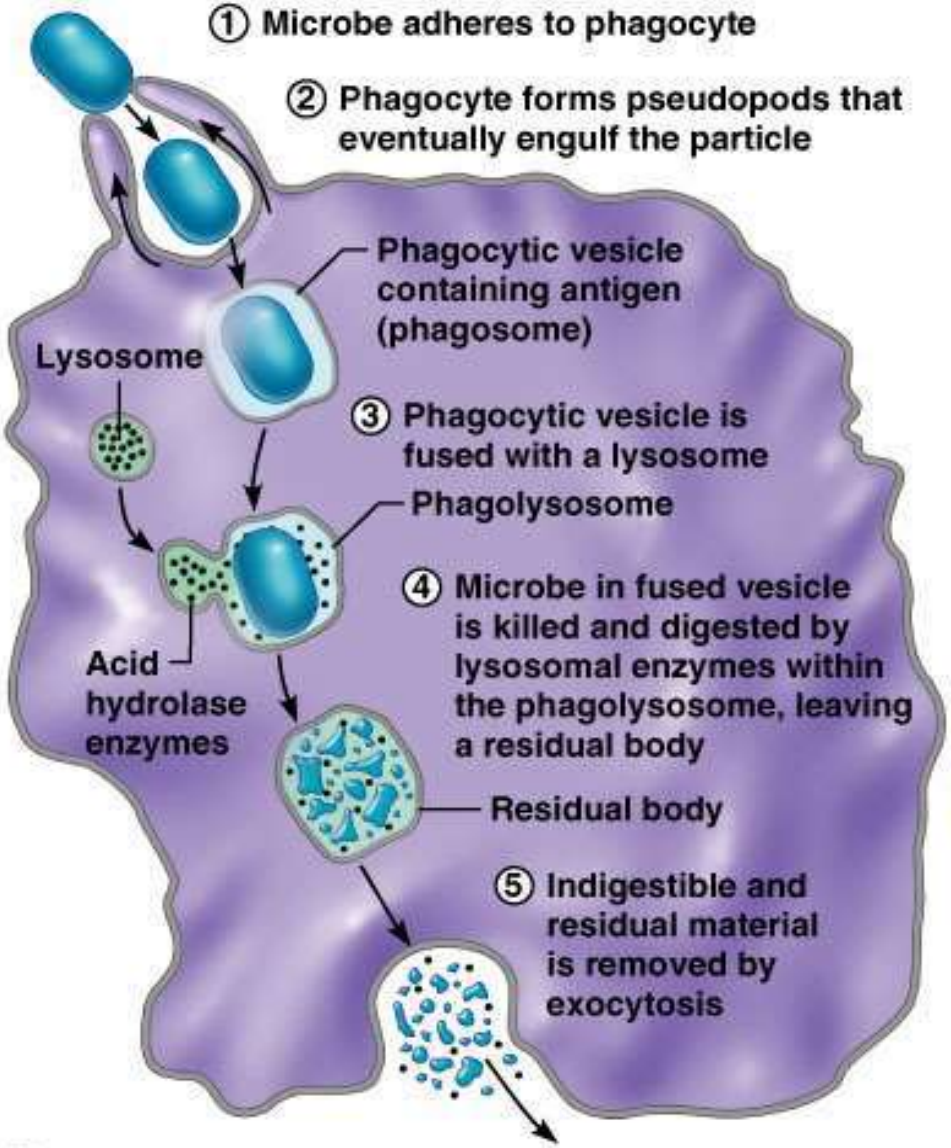


(b)

Chemotaxis & attachment

- Attraction by chemotactic substances (microbes, damaged tissues, complement components, vasoactive amines, etc)
- Attachment by receptors on surfaces of phagocytes.

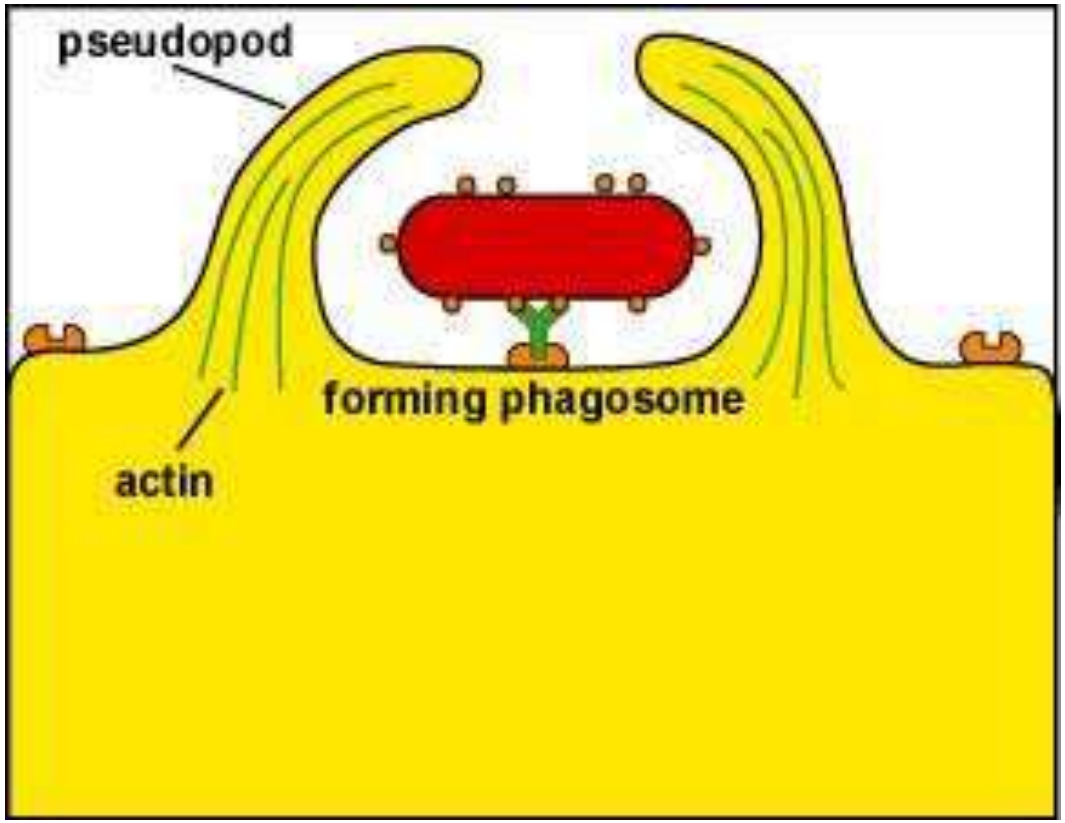
Ingestion and phagosome formation

- Phagocytes' produce pseudopodia surrounding organism forming phagosome
- Opsonins and co-factors enhance phagocytosis

Phagolysosome formation

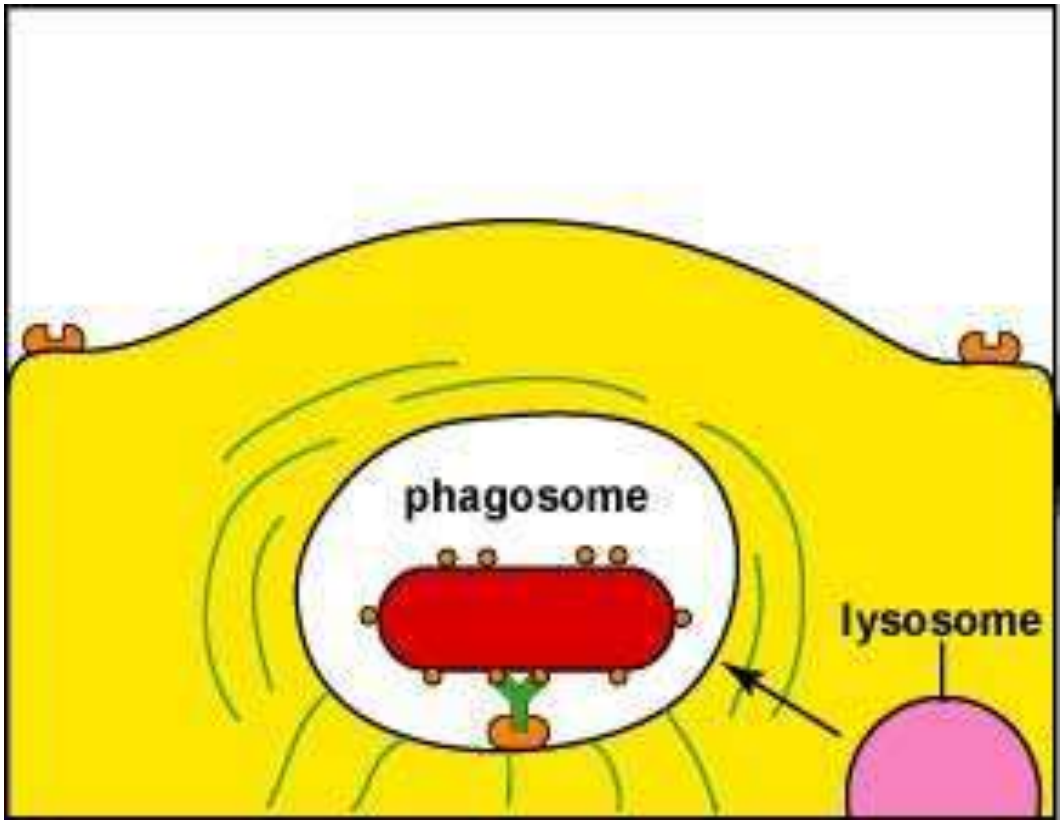
- Fusion of phagosome with lysosomal granules of phagocyte take place by help of cytoskeleton followed by the release digestive and degradative enzymes

The Process of Phagocytosis - ingestion



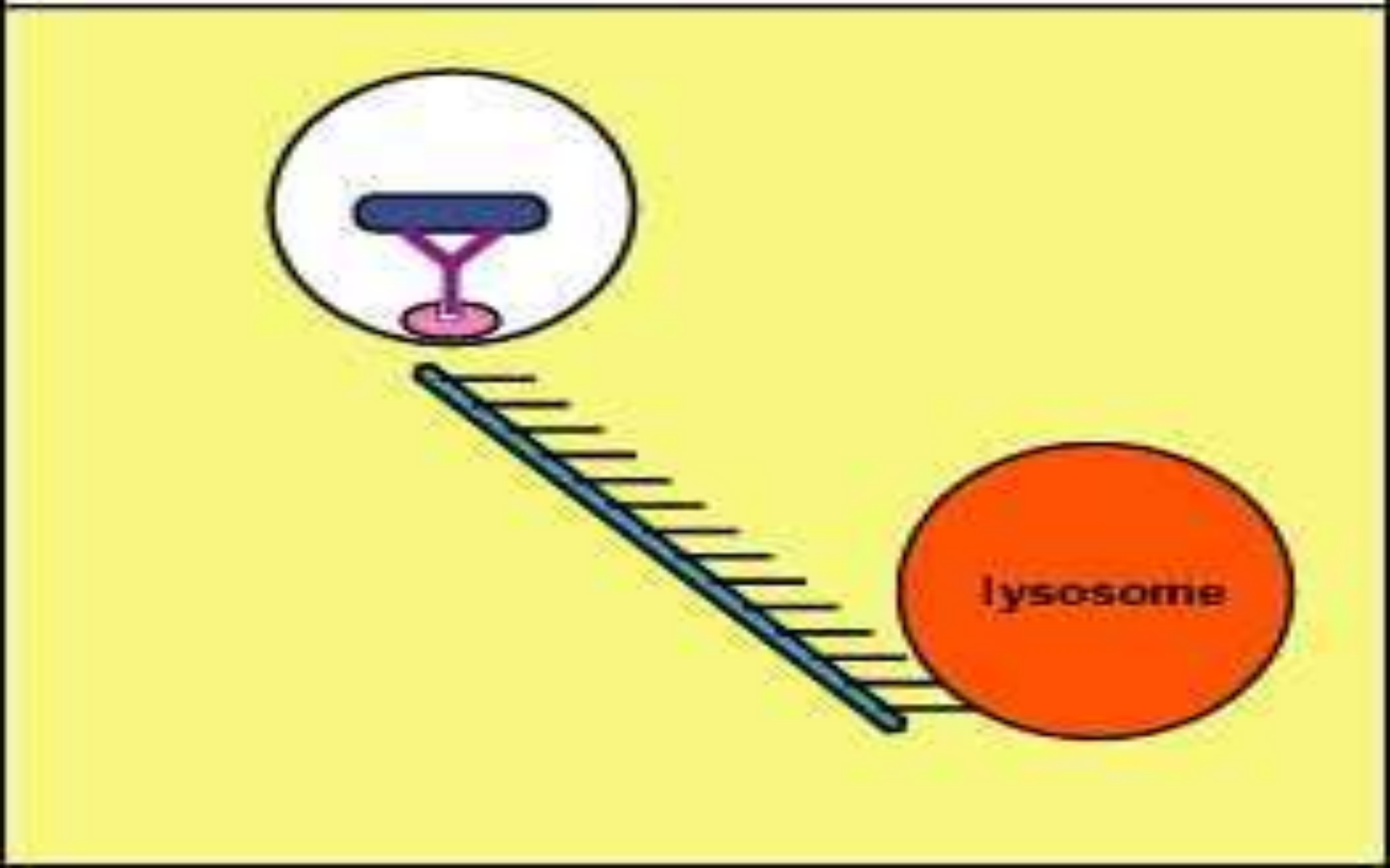
Following attachment, polymerization and depolymerization of actin molecules send pseudopods out to engulf the bacterium

The Process of Phagocytosis - phagosome formation

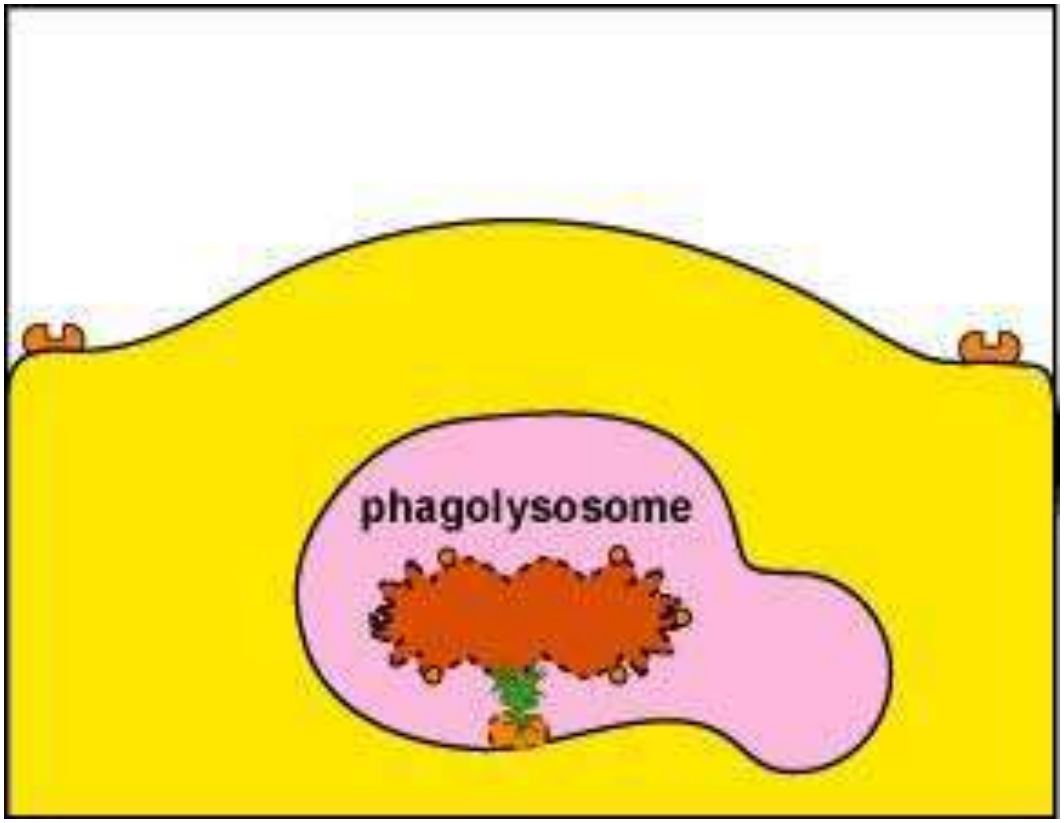


Following engulfment, the bacterium is placed in a vesicle called a phagosome.

Lysosomes move along the cytoskeleton and fuse with phagosomes to form phagolysosomes.



The Process of Phagocytosis - Destruction



The lysosome, its digestive enzymes and microbicidal chemicals fuses with the phagosome containing the ingested bacteria to form a phagolysosome and the bacterium is killed.

Intra-cellular killing (two microbicidal routes)

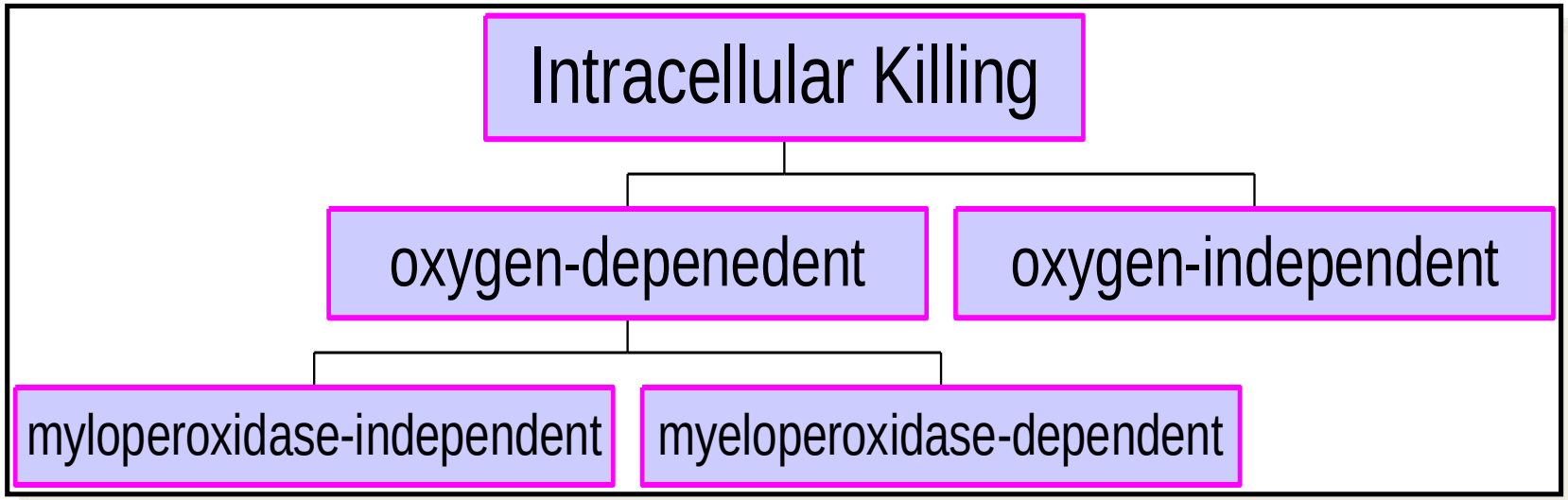
- **Oxygen-dependent system** (powerful microbicidal agents)

  Oxygen converted to superoxide anion, hydrogen peroxide, activated oxygen and hydroxyl radicals.

- **Oxygen-independent system** (anaerobic conditions)

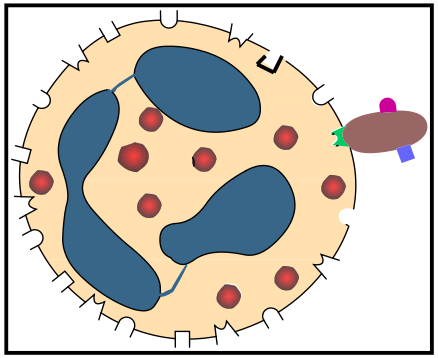
  Digestion and killing by lysozyme, lactoferrin, low pH, cationic proteins and hydrolytic and proteolytic enzymes

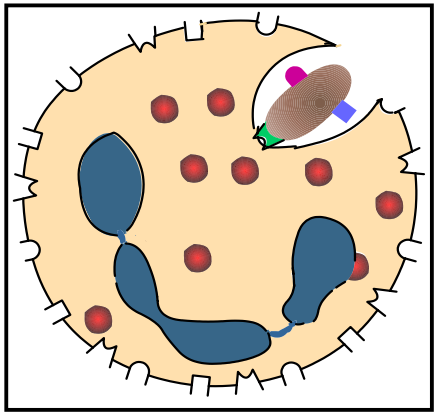
Pathways of Intracellular Killing



Respiratory Burst

Oxygen-dependent Myeloperoxidase-independent Reactions





$$\text{Glucose} + NADP^+ \xrightarrow[\text{G-6-P-dehydrogenase}]{} \text{Pentose-P} + NADPH$$

$$NADPH + O_2 \xrightarrow[\text{Cytochrome}_{b558}]{} NADP^+ + O_2^-$$

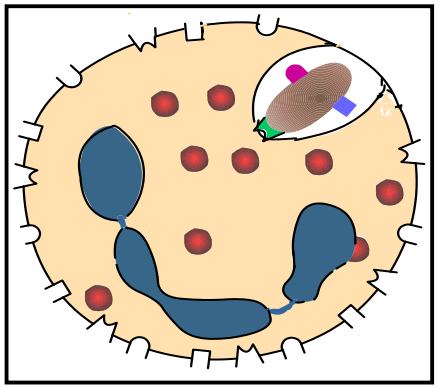
$$2O_2^- + 2H^+ \xrightarrow[\text{Superoxide dismutase}]{} H_2O_2 + {}^1O_2$$

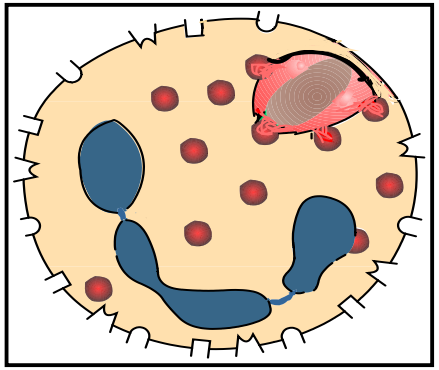
$$2O_2^- + H_2O_2 \rightarrow .OH + OH^- + {}^1O_2$$

Toxic compounds – Superoxide anion ($O2^-$), Hydrogen peroxide (H_2O_2), Singlet oxygen ($^1O_2$) and Hydroxyl radical (OH*)

Respiratory Burst

Oxygen-dependent Myeloperoxidase-dependent Reactions





$$H_2O_2 + Cl^- \xrightarrow[\text{myeloperoxidase}]{} OCl^- + H_2O$$

$$2OCL^- + H_2O \rightarrow {}^1O_2{}^- + Cl^- + H_2O$$

Toxic compounds

– Hypochlorous acid (OCl^-)

- Singlet oxygen ($^1O_2$)

Respiratory Burst

Detoxification Reactions

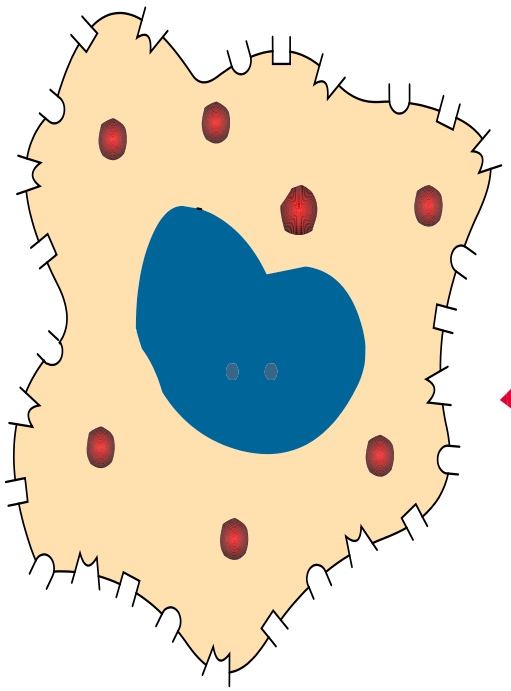
$$2O_2^- + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

Mediators of Oxygen-independent Killing in the Phagolysosome

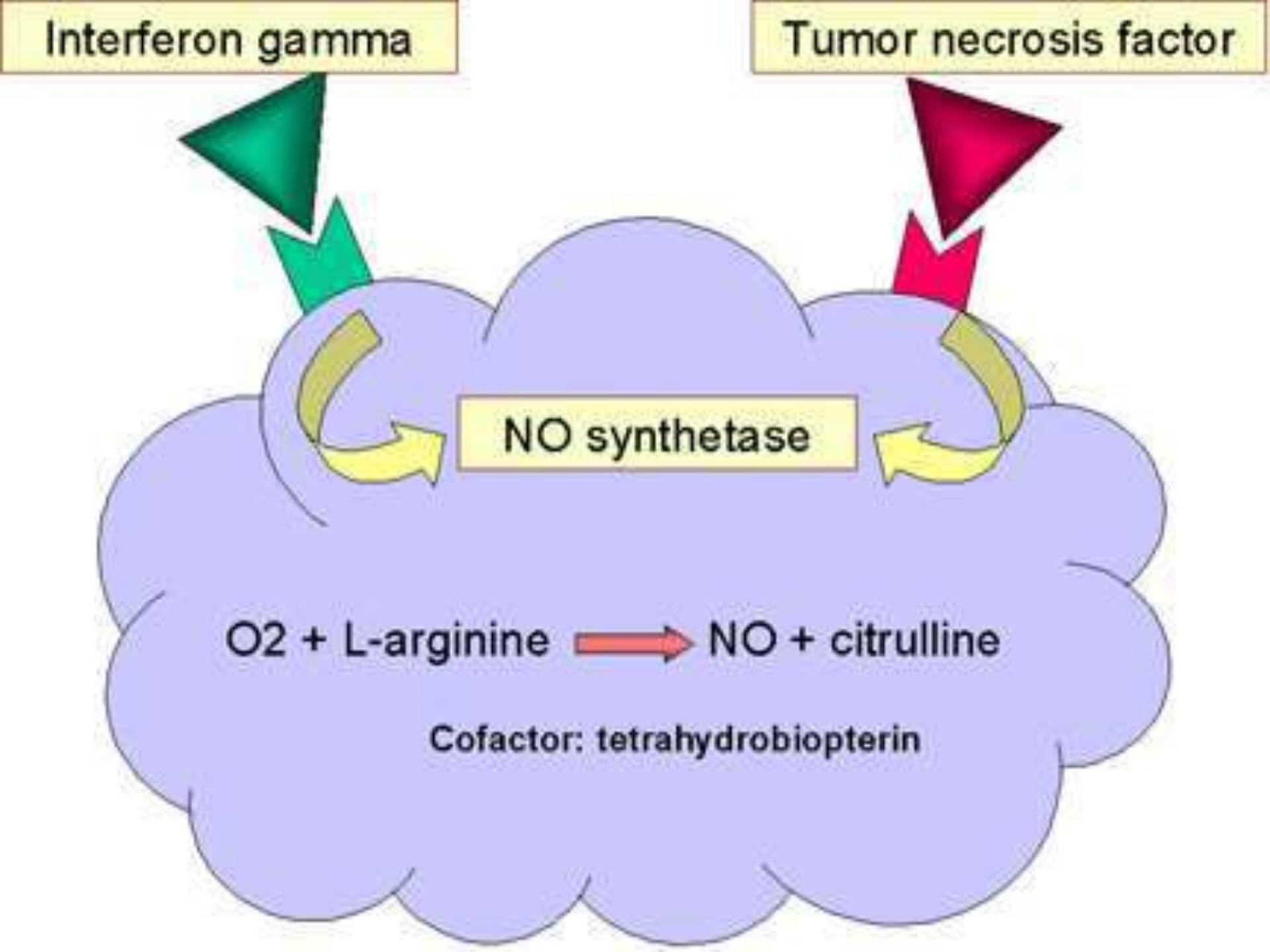
Effector Molecule	Function
Cationic proteins (cathepsin)	Damage to microbial membranes
Lysozyme	Hydrolyses mucopeptides in the cell wall
Lactoferrin	Deprives pathogens of iron
Hydrolytic enzymes (proteases)	Digests killed organisms

Nitric Oxide Dependent Killing



Nitric Oxide

Some cytokines can also induce phagocytic cells, particularly macrophages, to produce nitric oxide (NO), which is toxic to microorganisms and malignant cells

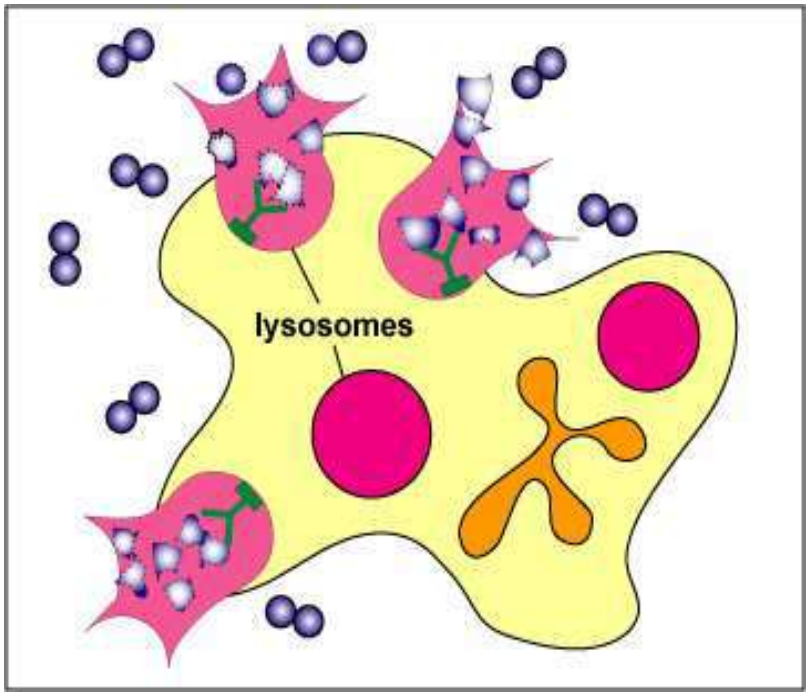

Interferon gamma
Tumor necrosis factor
NO synthetase
O2 + L-arginine
NO + citrulline
Cofactor: tetrahydrobiopterin

Nitric Oxide Dependent Killing

Nitric oxide also possess antiviral properties:

- inhibition of viral RNA synthesis
- inhibition of viral protein accumulation
- inhibition of virus release from infected cell

Extracellular Destruction of Bacteria by a Phagocyte



- If the phagocyte is overwhelmed with microorganisms, the phagocyte will empty the contents of its lysosomes by a process called degranulation in order to kill the microorganisms or cell extracellularly. These released lysosomal contents, however, also kill surrounding host cells and tissue. Most tissue destruction associated with infections is a result of this process

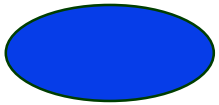
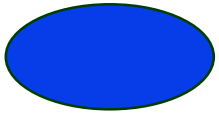
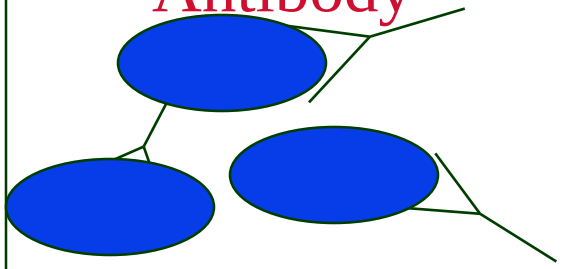
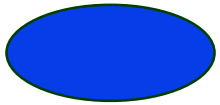
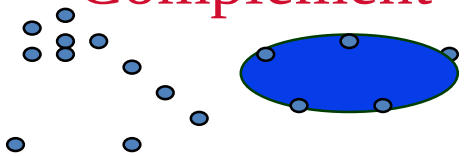
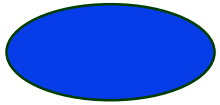
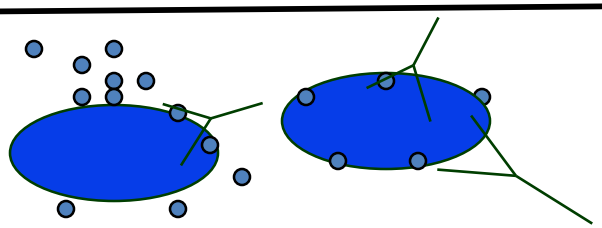
FACTORS AFFECTING PHAGOCYTOSIS

OPSONINS "natural ketchup"

Proteins which coat the antigen to facilitate phagocytosis

e.g. **Antibodies**
Complement Components
Certain Liver proteins

Generally both bacteria and cells that are suspended in body fluids have negative charges (Zeta Potential). Therefore they tend to repel each other. Opsonins, provide positive charges and coating with opsonins promotes phagocytosis.

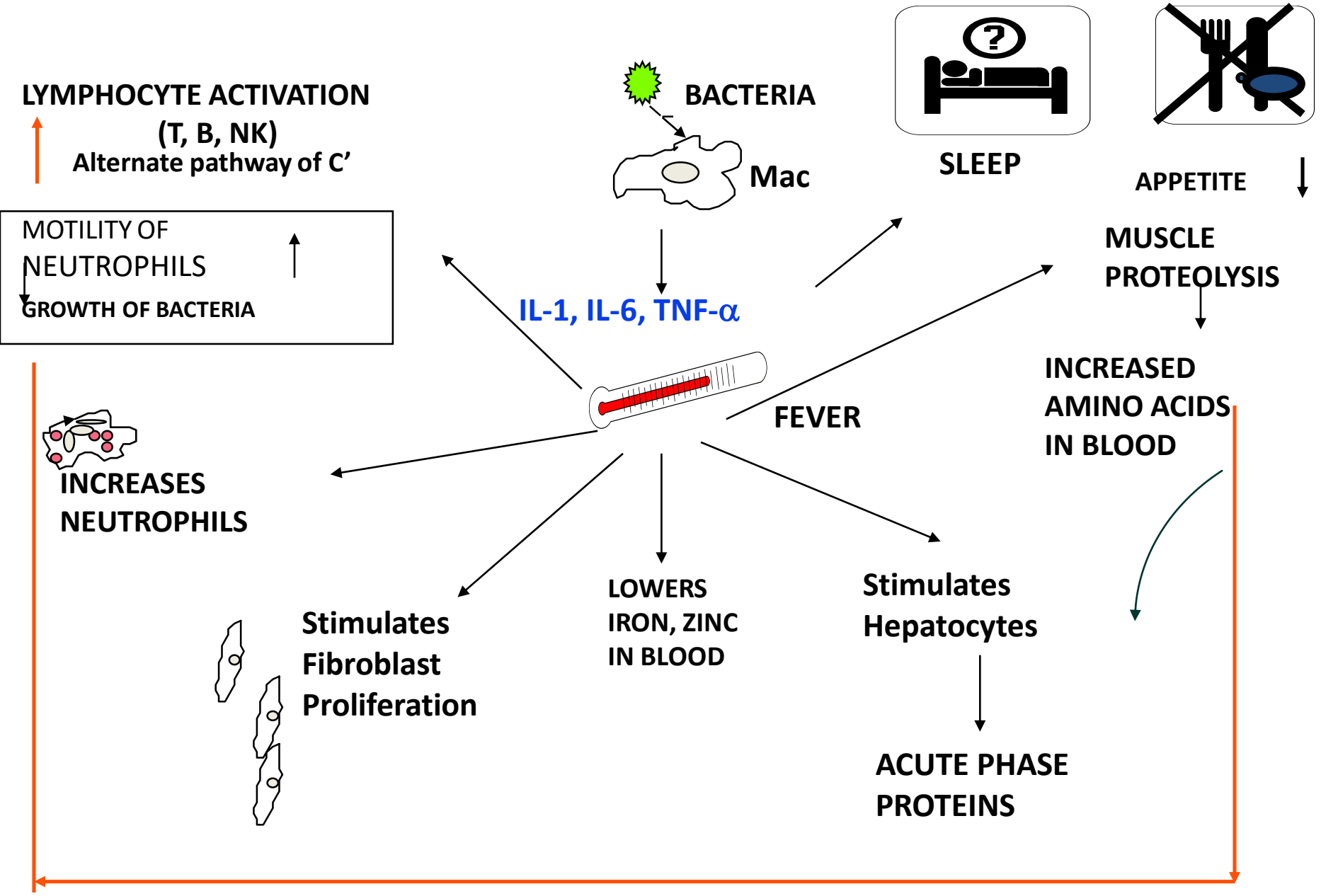
ANTIGEN	OPSONIN	EFFICIENCY OF PHAGOCYTOSIS
	None	+
	Antibody 	+++−
	Complement 	++−
	 Antibody Plus Complement	++++−

Fever

Fever

- Activated macrophages and other leukocytes release proinflammatory cytokines such as TNF-alpha, IL-6, and IL-1
- These cytokines stimulate the anterior hypothalamus of the brain to produce prostaglandins that lead to an increase in body temperature – **fever.**
- Fever increases the physiological temperature above the optimum growth temperature for many microorganisms.
- Fever leads to the production of heat shock proteins resulting in the production of inflammation-promoting cytokines.
- Fever elevates the temperature of the body increasing the rate of enzyme reactions, and speeding up metabolism within the body

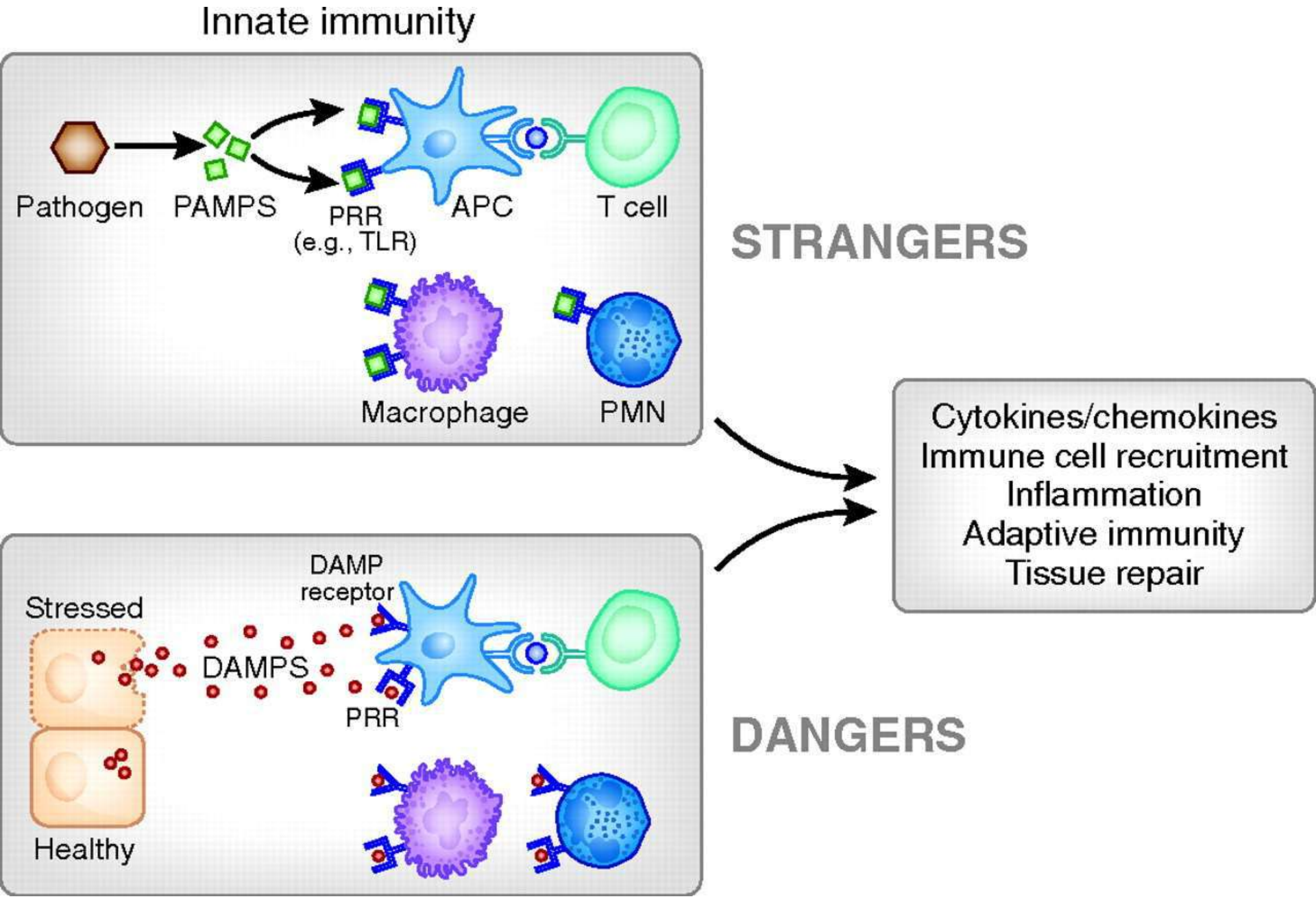
Fever and Immune Activation



How innate Immunity Recognizes?

Pattern-Recognition Receptors

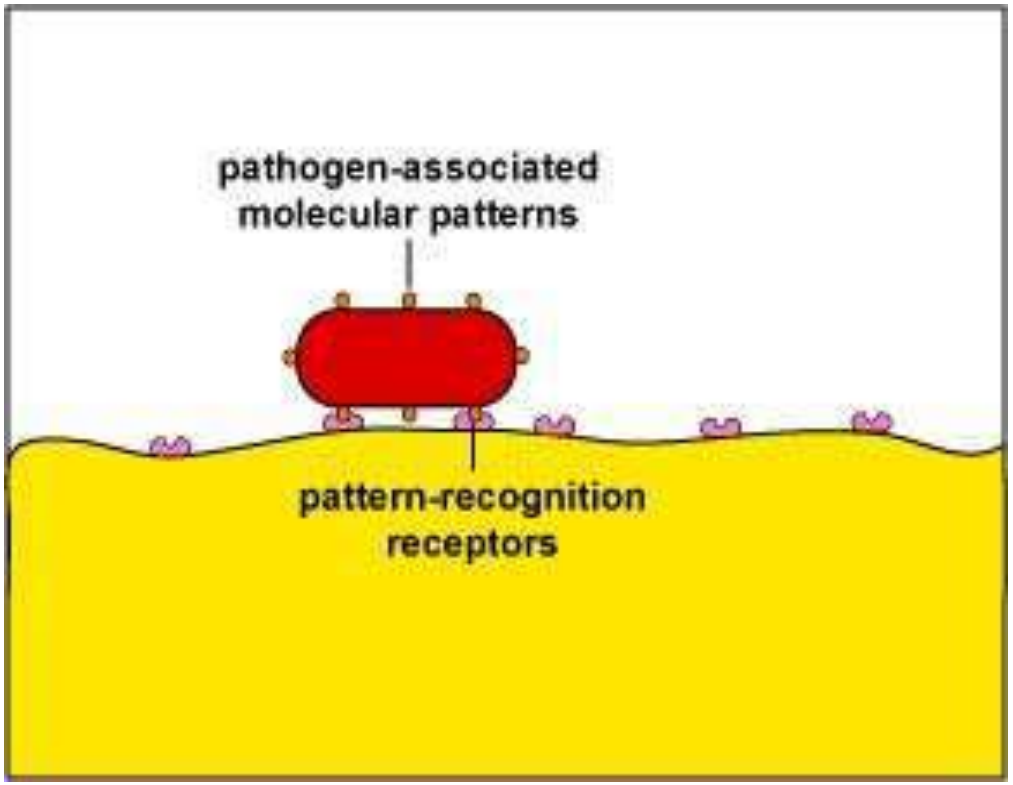
- Innate immunity recognize a few highly conserved structures present in many different microorganisms - the **pathogen-associated molecular patterns (PAMPs)** as well as **danger signals released from damaged or dying (necrotic) cells (DAMPs)**
- These PAMPs/DAMPs are recognized by **pattern recognition receptors (PRRs)**, expressed on/in the innate immunity cells.
- PRRs can also recognize host molecules containing **damage-associated molecular patterns (DAMPs),** molecules that are often released from necrotic cells damaged by invading pathogens
- These PRRs include Toll-like receptors **(TLRs)**, nucleotide-binding domain (NOD) and leucine-rich repeat containing receptors **(NLRs)**, and retinoic acid-inducible gene-I (RIG-)-like receptors **(RLRs)**. lectins, and scavenger receptors.


Innate immunity
Pathogen
PAMPS
PRR
(e.g., TLR)
APC
T cell
Macrophage
PMN
STRANGERS
Cytokines/chemokines
Immune cell recruitment
Inflammation
Adaptive immunity
Tissue repair
Stressed
DAMP
receptor
DAMPS
PRR
Healthy
DANGERS

PAMPs

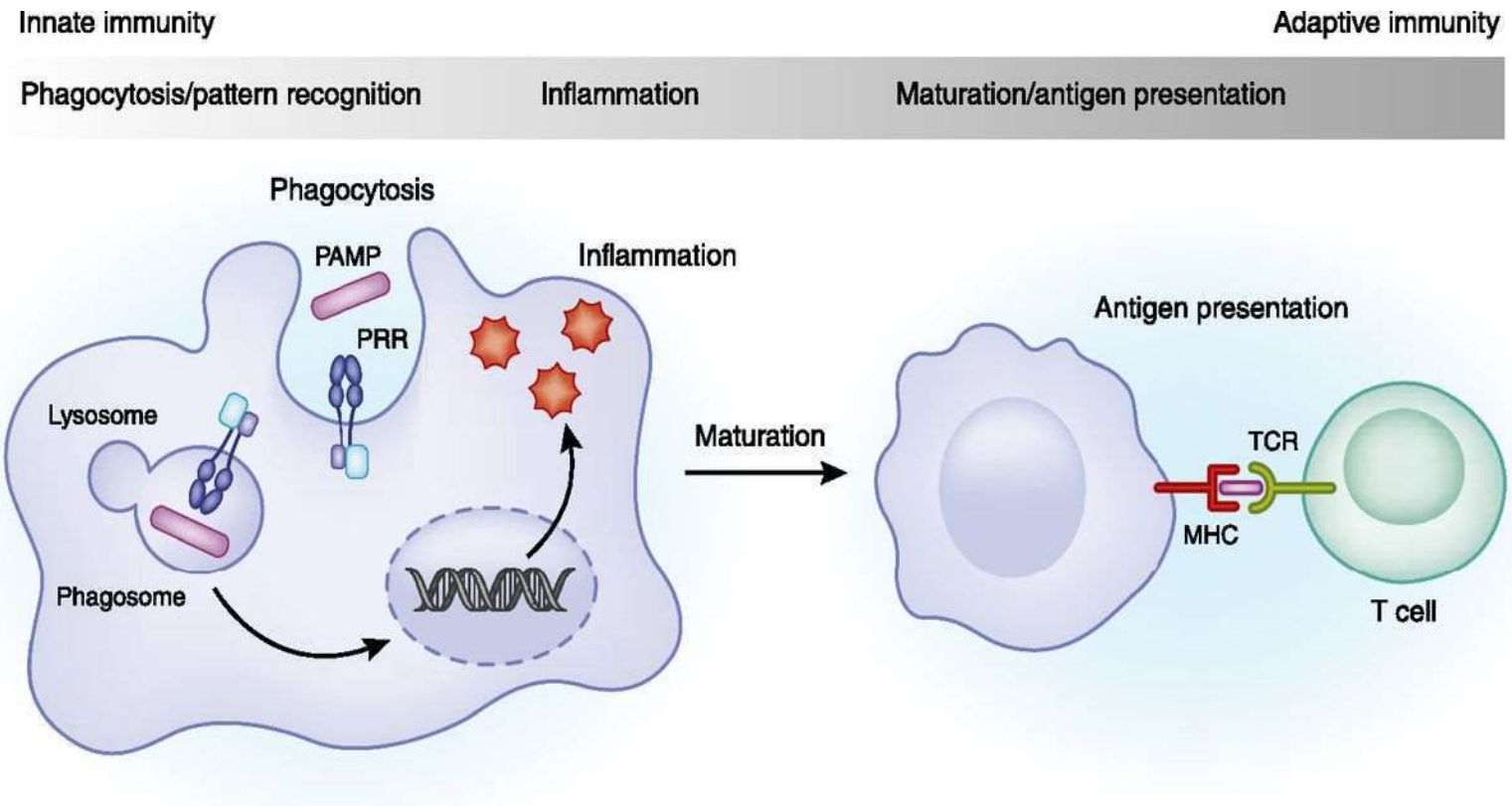
- Lipopolysaccharide
- Peptidoglycan
- Lipoteichoic acids
- Mannose-rich glycans
- Flagellin
- Pilin
- Bacterial nucleic acid
- N-formylmethionine,
- Double-stranded RNA
- Lipoteichoic acids, glycolipids, and zymosan
- Phosphorylcholine and other lipids

PAMPs binding to PRRs on defense cells



The PRRs recognize approximately 10^3 molecular patterns

Innate immune responses encountered by microbes.



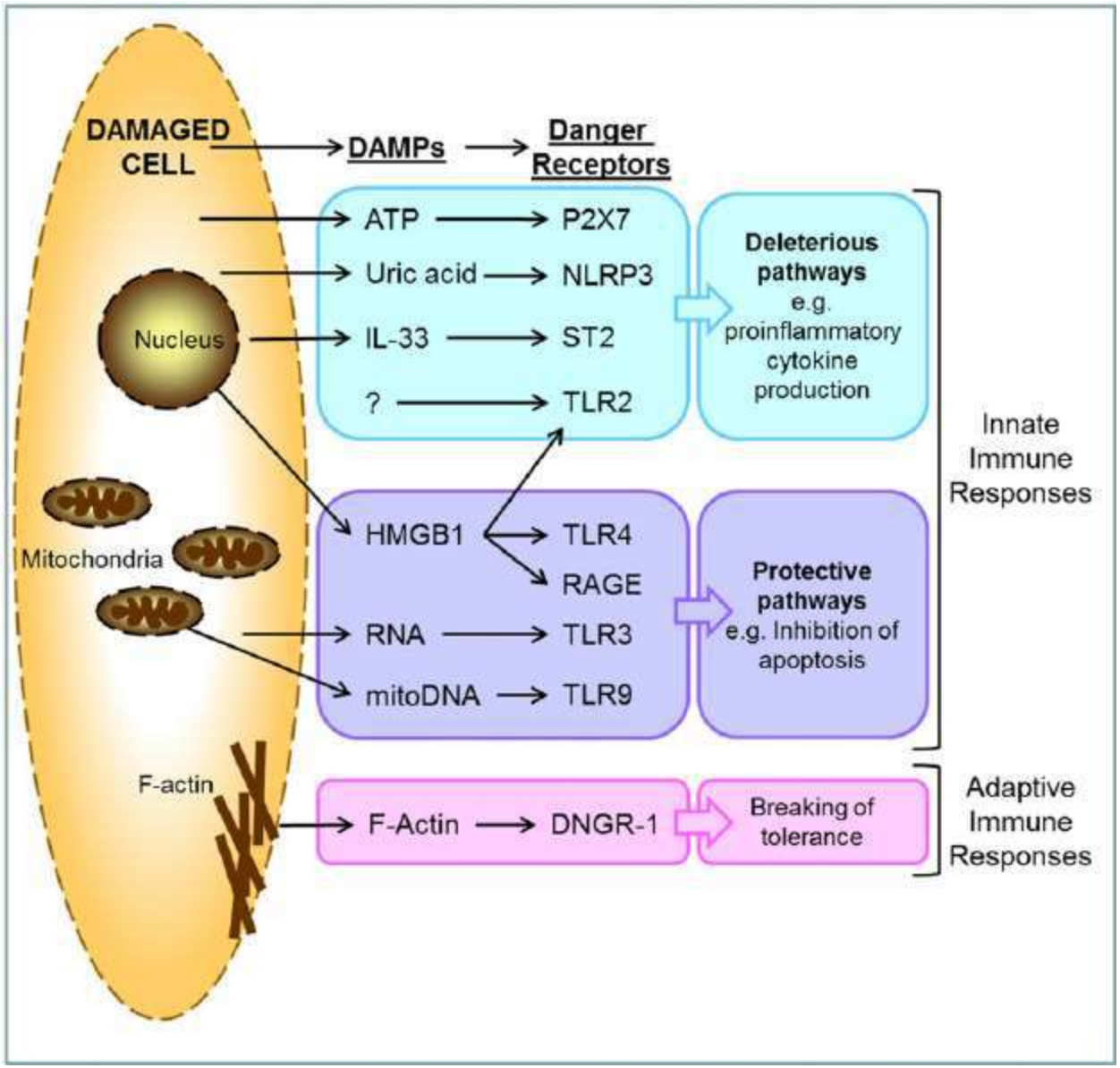
Microbes are detected by pattern recognition receptors (PRRs) expressed in innate immune cells, such as macrophages. The detection of microbes by the PRRs rapidly activates signalling cascades and generates inflammatory responses. Microbial encounter also leads to maturation of macrophages and dendritic cells into antigen presenting cells. PAMP, pathogen-associated molecular pattern; TCR, T-cell receptor.

PAMP	PRR	Biological Consequence of Interaction
Microbial cell wall components	Complement	Opsonization; Complement activation
Mannose-containing carbohydrates	Mannose-binding protein	Opsonization; Complement activation
Lipoproteins of Gram positive bacteria, yeast cell wall components	TLR-2 (Toll-like receptor 2)	Macrophage activation; Secretion of inflammatory cytokines
Double stranded RNA	TLR-3	Production of interferon (antiviral)
LPS (lipopolysaccharide of Gram –ve bacteria	TLR-4	Macrophage activation; Secretion of inflammatory cytokines
Flagellin (bacterial flagella)	TLR-5	Macrophage activation; Secretion of inflammatory cytokines

DAMPs

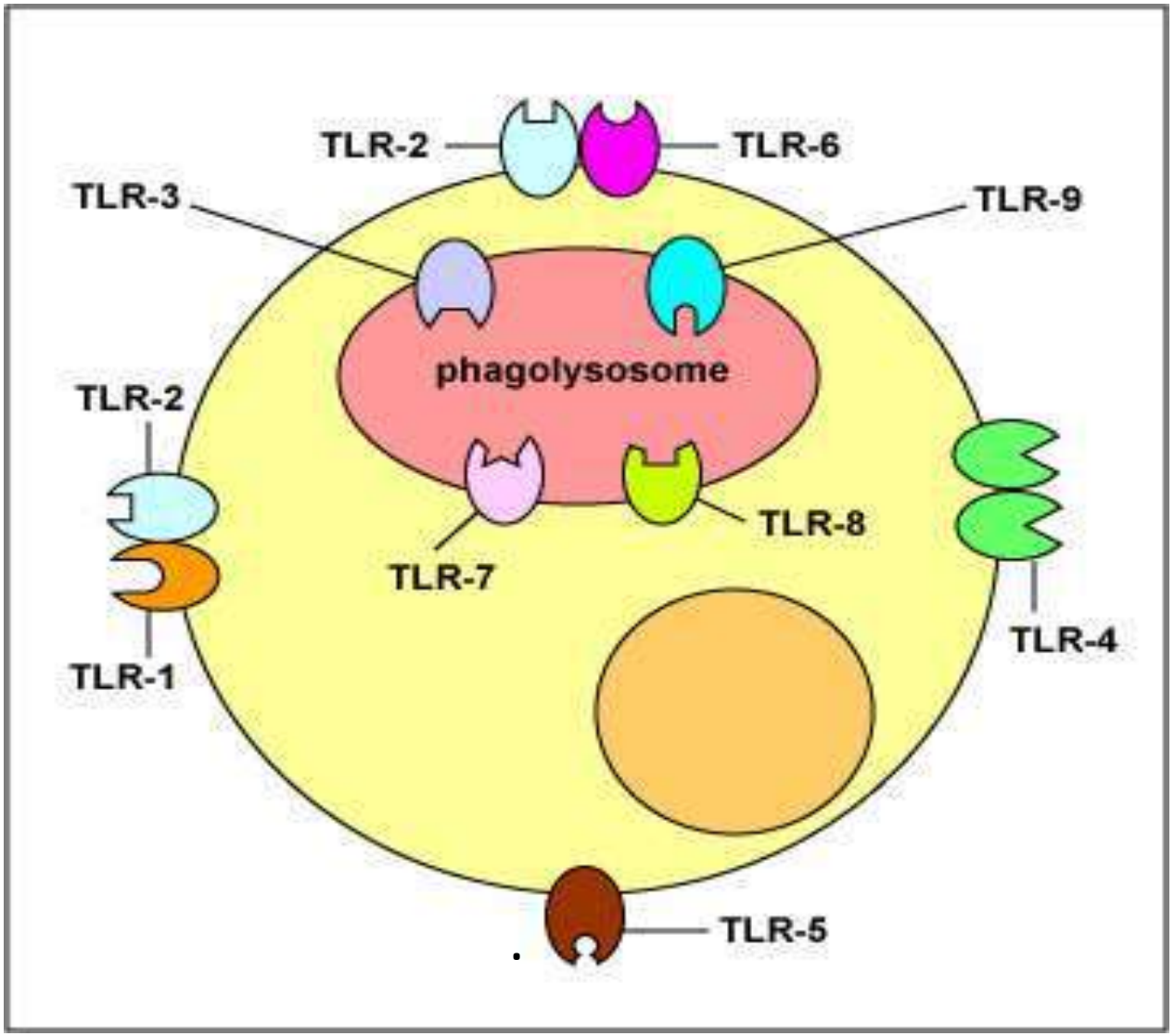
- Damage-associated molecular patterns (DAMPs) are endogenous danger molecules that are released released upon cellular stress or tissue injury from damaged or dying cells and activate the innate immune system by interacting with pattern recognition receptors (PRRs).
- DAMPs activate the innate immune system by inducing potent inflammatory responses during non-infectious inflammation
- These DAMPs are recognized by macrophages, and inflammatory responses are triggered by different pathways, including TLRs and inflammasomes
- DAMPs can originate from different sources and include:
  - extracellular proteins, e.g. biglycan and tenascin C,
  - intracellular proteins, e.g. high-mobility group box 1 (HMGB1), histones, S100 proteins, heat-shock proteins (HSPs), and
  - plasma proteins, e.g. fibrinogen, Gc-globulin, and serum amyloid A (SAA)

Induction of immune response by DAMPs



Toll-like receptors (TLRs) - 1

- **Play a major role in innate immunity and the induction of adaptive immunity**.
- Different combinations of TLRs appear in different cell types and seem to appear in pairs; 13 recognized so far
- Different TLRs directly or indirectly bind different microbial molecules.
- **TLRs are found both on the surface and within the phagolysosomes of phagocytes.**
- Surface TLRs recognize molecules on the surface of microbes such as cell wall components
- Internal TLRs recognize microbial molecules released upon phagocytosis of the microbe.


TLR-2
TLR-6
TLR-3
TLR-9
phagolysosome
TLR-2
TLR-8
TLR-7
TLR-4
TLR-1
TLR-5

Consequences of TLR Signal Transduction

NFκB Activation → Transcriptional Activation

Transcriptional Activation → Synthesis of:

- ROIs
- Anti-microbial peptides
- Cytokines
- Chemokines
- Adhesion molecules
- Acute phase proteins