Chemotaxis

- After extravasation, leukocytes emigrate in tissues toward the site of injury by a process called *chemotaxis*, defined most simply as locomotion oriented along a chemical gradient.
- All granulocytes, monocytes and, to a lesser extent, lymphocytes respond to chemotactic stimuli with varying rates of speed.
- Both exogenous and endogenous substances can act as chemoattractants.
  - The most common *exogenous* agents are *bacterial products*. Some of these are peptides that possess an *N*-formyl-methionine terminal amino acid. Others are lipid in nature.
  - *Endogenous* chemoattractants, which are detailed later, include several chemical mediators:

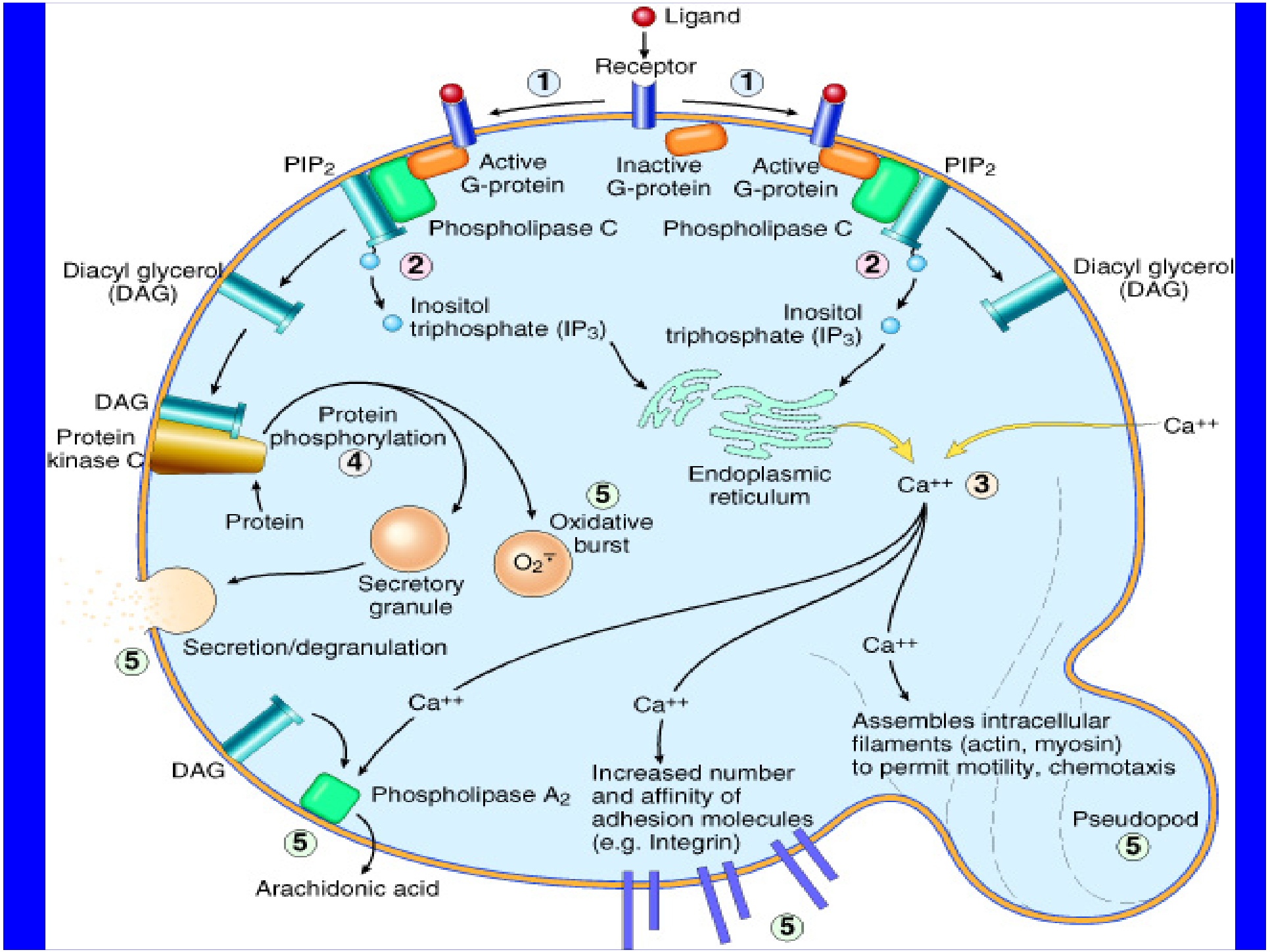
(1) components of the complement system, particularly C5a

(2) products of the lipoxygenase pathway, mainly leukotriene B_4 (LTB_4)

(3) cytokines, particularly those of the chemokine family (e.g., IL-8).

Chemotaxis

- All these chemotactic agents bind to specific seven-transmembrane G-protein-coupled receptors (GPCRs) on the surface of leukocytes.
- Signals initiated from these receptors result in recruitment of G-proteins and activation of several effector molecules, including phospholipase C (PLCγ) and phosphoinositol-3 kinase (PI3K), and protein tyrosine kinases.
- PLCγ and PI3K act on membrane inositol phospholipids to generate lipid second messengers that increase cytosolic calcium and activate small GTPases as well as numerous kinases.
- The GTPases induce polymerization of actin, resulting in increased amounts of polymerized actin at the leading edge of the cell. The leukocyte moves by extending filopodia that pull the back of the cell in the direction of extension


Ligand
Receptor
1
1
PIP2
Active G-protein
Inactive G-protein
Active G-protein
PIP2
Phospholipase C
Phospholipase C
2
2
Diacyl glycerol (DAG)
Diacyl glycerol (DAG)
Inositol triphosphate (IP3)
Inositol triphosphate (IP3)
DAG
Protein kinase C
Protein phosphorylation
4
Protein
Endoplasmic reticulum
Ca++
Ca++ 3
5
Oxidative burst
O2-
Secretory granule
Secretion/degranulation
5
Ca++
Ca++
Ca++
DAG
Phospholipase A2
5
Arachidonic acid
Increased number and affinity of adhesion molecules (e.g. Integrin)
Assembles intracellular filaments (actin, myosin) to permit motility, chemotaxis
Pseudopod
5
5

Leukocyte Activation

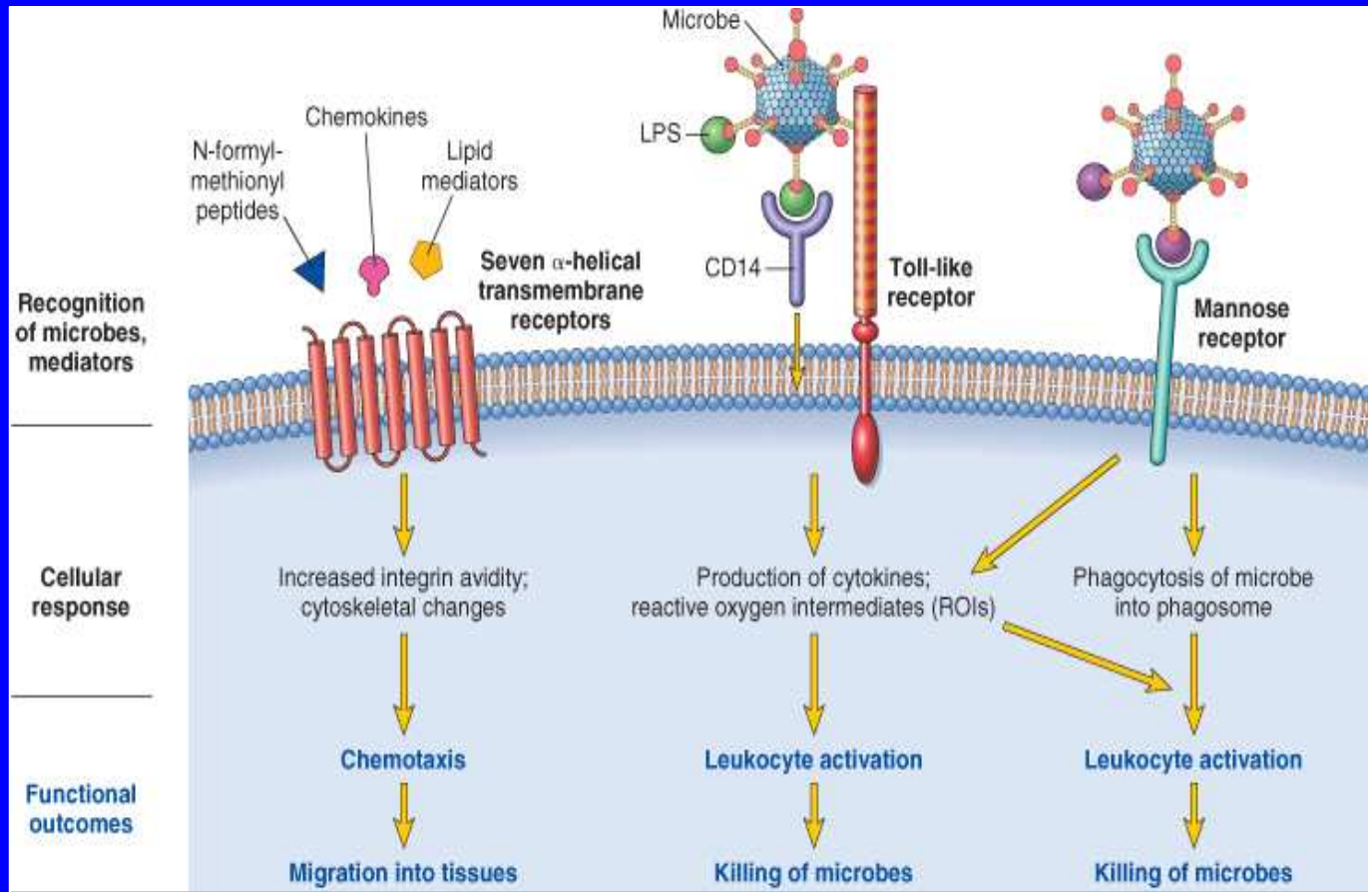
- Microbes, products of necrotic cells, antigen-antibody complexes, and cytokines, including chemotactic factors, induce a number of responses in leukocytes that are part of the defensive functions of the leukocytes (neutrophils and monocytes/macrophages)
- Activation results from several signaling pathways that are triggered in leukocytes, resulting in increases in cytosolic Ca^{2+} and activation of enzymes such as protein kinase C and phospholipase A_2.

Leukocyte activation

- The functional responses that are induced on leukocyte activation include the following:
  - *Production of arachidonic acid metabolites* from phospholipids, as a result of activation of phospholipase A_2 by increased intracellular calcium and other signals.
  - *Degranulation and secretion of lysosomal enzymes and activation of the oxidative burst*
  - *Secretion of cytokines*, which amplify and regulate inflammatory reactions.
    - Activated macrophages are the chief source of the cytokines
  - *Modulation of leukocyte adhesion molecules*.
    - different cytokines cause increased endothelial expression of adhesion molecules and increased avidity of leukocyte integrins, allowing firm adhesion of activated neutrophils to endothelium

Leukocyte activation

Toll-like receptors
Different seven-transmembrane G-protein-coupled receptors
receptors for cytokines
Receptors for opsonins

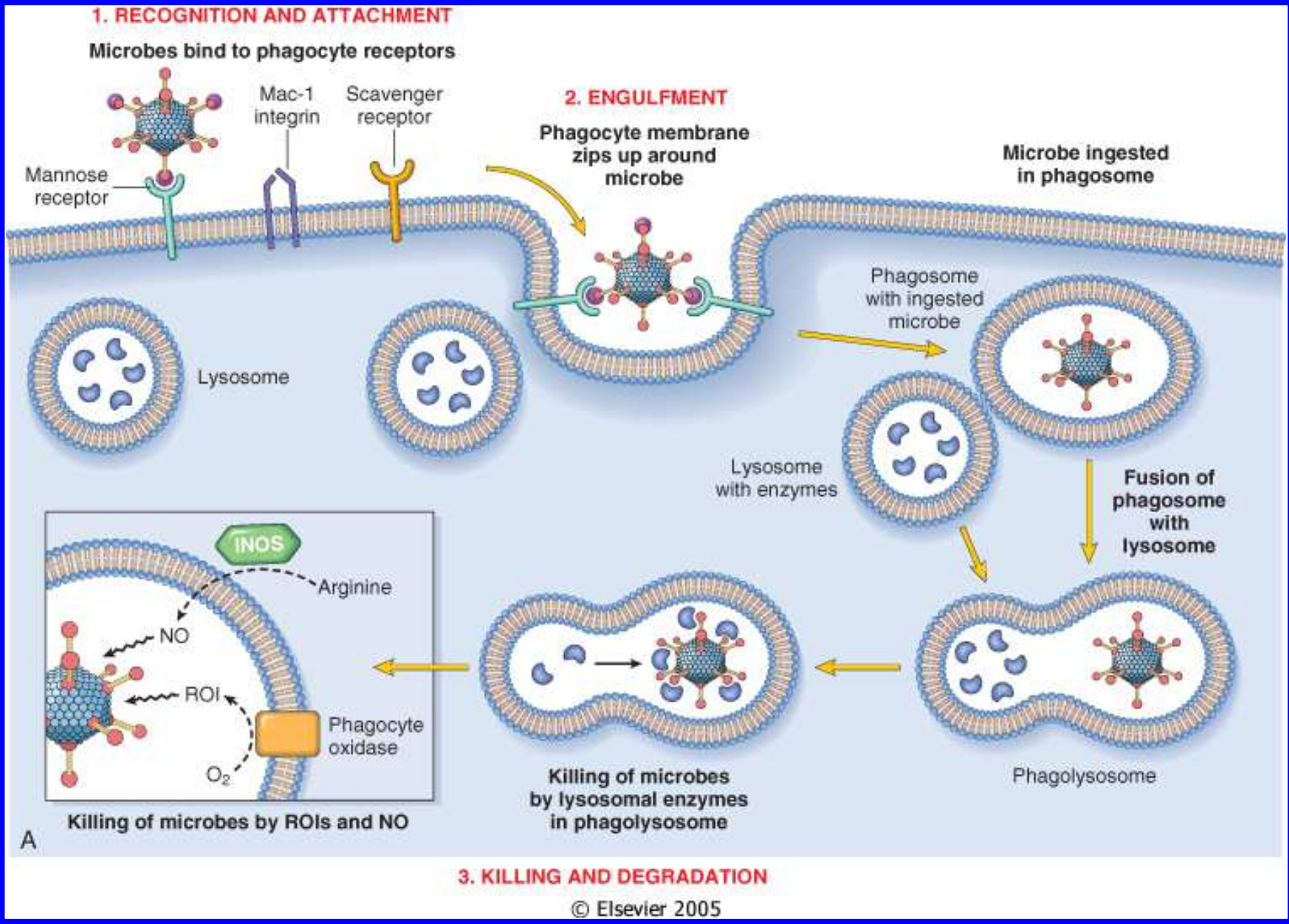


Leukocyte activation

- *Receptors for opsonins*
- The process of coating a particle, such as a microbe, to target it for phagocytosis is called ***opsonization***, and substances that do this are *opsonins*.
- These substances include antibodies (IgG), complement proteins (C3), and lectins (mannose-binding lectin (MBL), fibronectin, fibrinogen, and C-reactive protein),
- These can coat microbes and are recognized by receptors on phagocytes.

Phagocytosis

- Phagocytosis and the release of enzymes by neutrophils and macrophages are responsible for eliminating the injurious agents
- Phagocytosis involves three distinct but interrelated steps
  - (1) *recognition* and *attachment* of the particle to be ingested by the leukocyte
  - (2) its *engulfment*, with subsequent formation of a phagocytic vacuole
  - (3) *killing* or *degradation* of the ingested material.



Phagocytosis

Recognition and Attachment

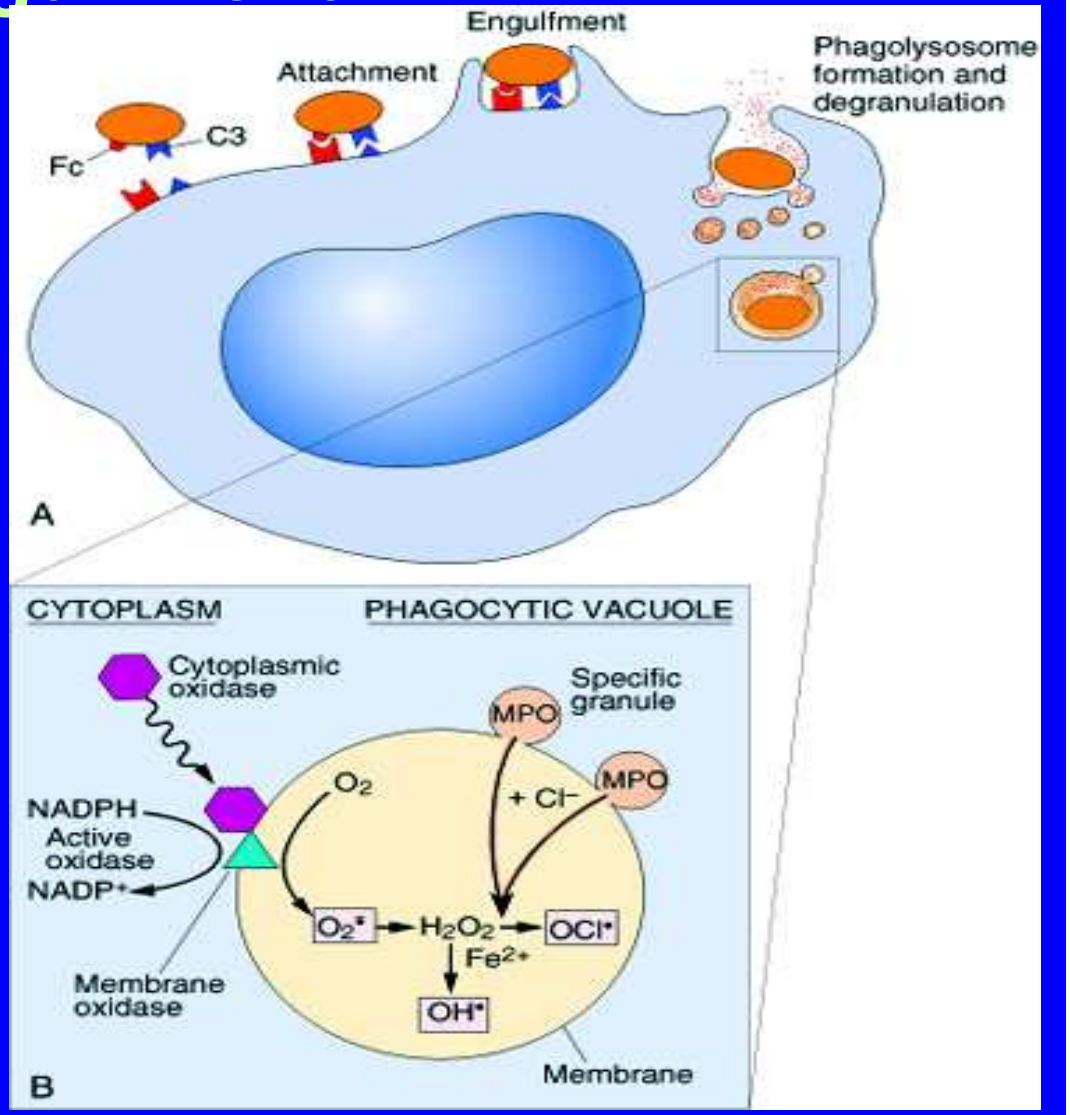
- Phagocytosis of microbes and dead cells is initiated by recognition of the particles by receptors expressed on the leukocyte surface:
- *Mannose receptors*
  - is a macrophage lectin that binds terminal mannose and fucose residues of glycoproteins and glycolipids.
  - These sugars are typically part of molecules found on microbial cell walls, whereas mammalian glycoproteins and glycolipids contain terminal sialic acid or *N*-acetylgalactosamine.
- *Scavenger receptors*
  - bind a variety of microbes in addition to modified LDL particles.
  - Macrophage integrins may also bind microbes for phagocytosis.

The efficiency of phagocytosis is greatly enhanced when microbes are opsonized

Phagocytosis

Engulfment

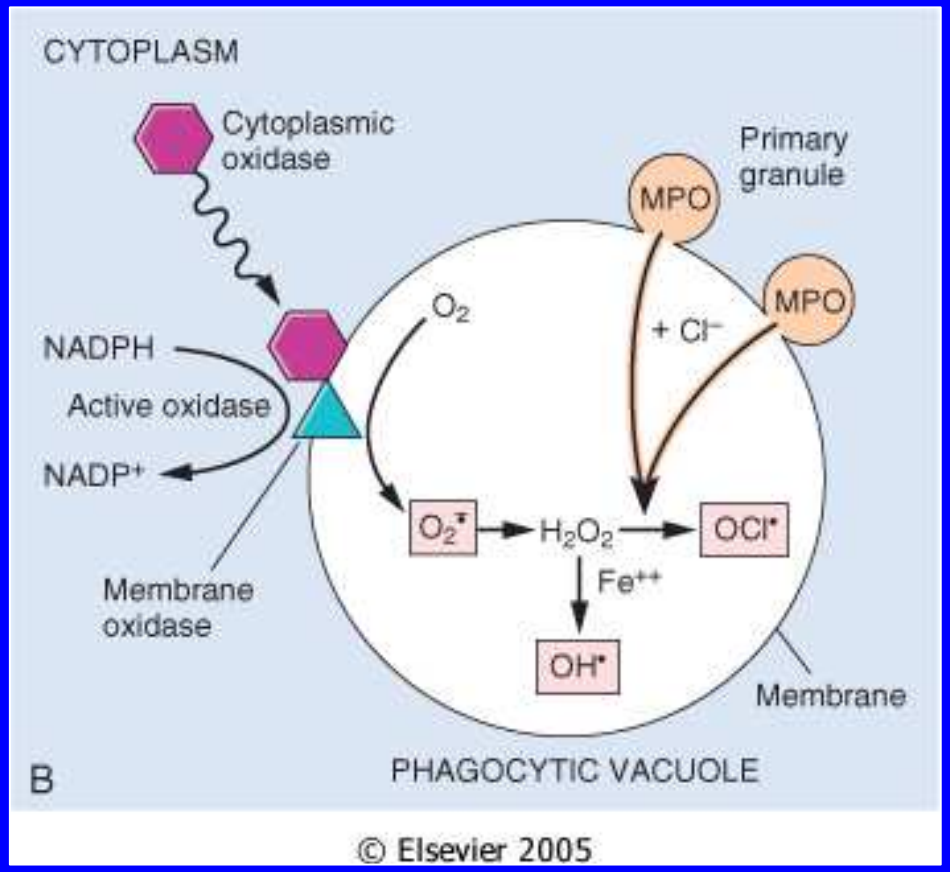
- During engulfment, extensions of the cytoplasm (pseudopods) flow around the particle to be engulfed, eventually resulting in complete enclosure of the particle within a phagosome
- The limiting membrane of this phagocytic vacuole then fuses with the limiting membrane of a lysosomal granule, resulting in discharge of the granule's contents into the phagolysosome
- During this process, the neutrophil and the monocyte become progressively degranulated.



Phagocytosis

Killing and Degradation

- *Microbial killing is accomplished largely by oxygen-dependent mechanisms*
- is due to the rapid activation of an oxidase (NADPH oxidase), which oxidizes NADPH (reduced nicotinamide-adenine dinucleotide phosphate) and, in the process, reduces oxygen to superoxide anion. Superoxide is then converted into hydrogen peroxide
- (H_2O_2) combine with azurophilic granules of neutrophils, contain the enzyme *myeloperoxidase* (MPO), which, in the presence of a halide such as Cl^-, converts H_2O_2 to hypochlorite (HOCl).
- hypochlorite is potent antimicrobial agent that destroys microbes by *halogenation* or by oxidation of proteins and lipids (lipid peroxidation).



•The H_2O_2-MPO-halide system is the most efficient bactericidal system in neutrophils

Phagocytosis

Killing and Degradation

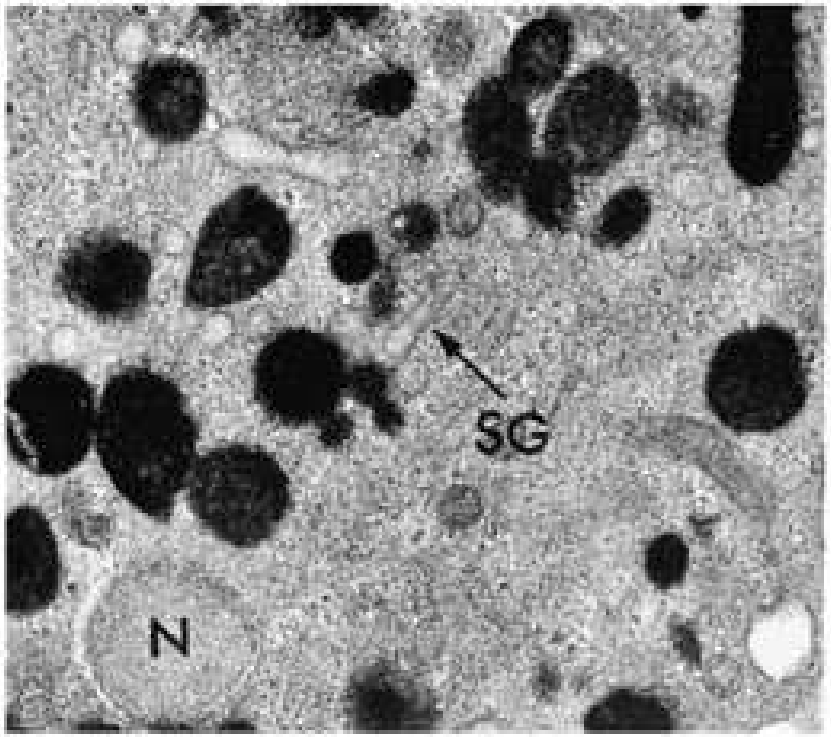
- *Oxygen-independent mechanisms*
- through the action of substances in leukocyte granules. These include:
  - *bactericidal permeability increasing protein* (BPI), a highly cationic granule-associated protein that causes phospholipase activation, phospholipid degradation, and increased permeability in the outer membrane of the microorganisms
  - *lysozyme*, which hydrolyzes the muramic acid-*N*-acetyl-glucosamine bond, found in the glycopeptide coat of all bacteria
  - *lactoferrin*, an iron-binding protein present in specific granules
  - *major basic protein*, a cationic protein of eosinophils, which has limited bactericidal activity but is cytotoxic to many parasites
  - *defensins*, cationic arginine-rich granule peptides that are cytotoxic to microbes (and certain mammalian cells).

- In addition, neutrophil granules contain many *enzymes*, such as elastase, that also contribute to microbial killing

Phagocytosis

- During activation and phagocytosis, leukocytes release microbicidal and other products not only within the phagolysosome but also into the extracellular space.
- The most important of these substances in neutrophils and macrophages are *lysosomal enzymes*, present in the granules; *reactive oxygen intermediates*; and *products of arachidonic acid metabolism,* including prostaglandins and leukotrienes.
- These products are capable of causing endothelial injury and tissue damage and may thus amplify the effects of the initial injurious agent

- After phagocytosis, neutrophils rapidly undergo *apoptotic cell death* and are ingested by macrophages



SPECIFIC GRANULES

- Lactoferrin
- Lysozyme
- Alkaline phosphatase
- Type IV collagenase
- Leukocyte adhesion molecules
- Plasminogen activation
- Phospholipase A_2

AZUROPHIL GRANULES

- Myeloperoxidase
- Lysozyme ← Bactericidal factors
- Cationic proteins
- Acid hydrolases
- Elastase
- Nonspecific collagenase
- BPI
- Defensins
- Cathepsin G
- Phospholipase A_2

Phagocytosis

In summary

- During activation and phagocytosis, leukocytes release microbicidal and other products not only within the phagolysosome but also into the extracellular space.
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