

Intracellular Destruction of Microbes and Debris

Leukocyte microbicidal mechanisms in acute inflammation

From recognition and engulfment to intracellular killing and collateral injury

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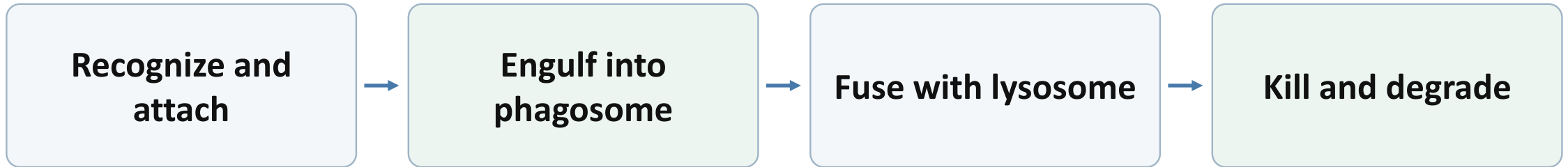
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Learning objectives

- Relate phagocytosis to the intracellular killing of microbes and dead-cell debris.
- Explain the NADPH oxidase–ROS and iNOS–reactive nitrogen pathways.
- Distinguish the functions of neutrophil granules, antimicrobial proteins, and antiproteases.
- Predict how defective killing or extracellular release causes disease.

Focus: Mechanism → cellular compartment → consequence

Intracellular destruction completes phagocytosis

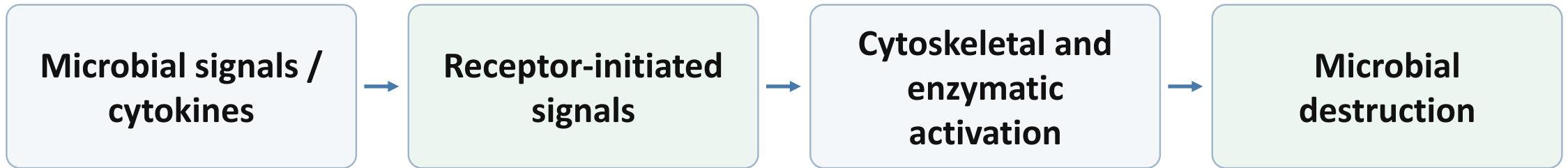


- The final step removes infectious agents and necrotic debris.
- Most efficient killing follows leukocyte activation.

Two professional phagocytes, complementary responses

Property	Neutrophils	Macrophages
Speed	Rapid; short-lived	Slower; more prolonged
Key oxidant response	Prominent respiratory burst	Reactive nitrogen species prominent
Granule release	Major response	Less prominent
Cytokine production	Limited	Major functional response

Leukocyte activation makes intracellular killing effective



- Activation coordinates ingestion with the microbicidal apparatus.
- Phagocytosis and killing are distinct but linked outputs.

Phagocytosis has three linked phases

Recognition

Microbe or debris engages receptors on the leukocyte.

Engulfment

Actin-driven membrane extension creates a phagosome.

Destruction

Lysosomal fusion brings toxic molecules to the cargo.

Direct recognition of microbial surfaces

Pattern-binding receptors

- Mannose receptor binds terminal mannose/fucose.
- Scavenger receptors bind microbes and modified lipoproteins.

Additional binding route

- Mac-1 integrin (CD11b/CD18) may bind microbes.
- Different receptor classes support particle attachment.

Why the mannose receptor discriminates microbes

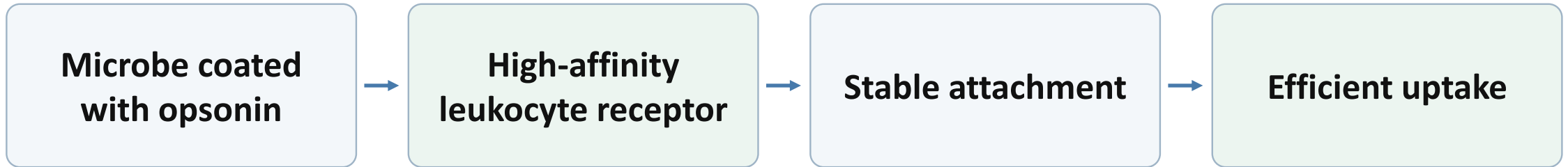
Microbial surfaces

- Terminal mannose and fucose residues are common.
- Macrophage lectin receptor recognizes these sugars.

Mammalian surfaces

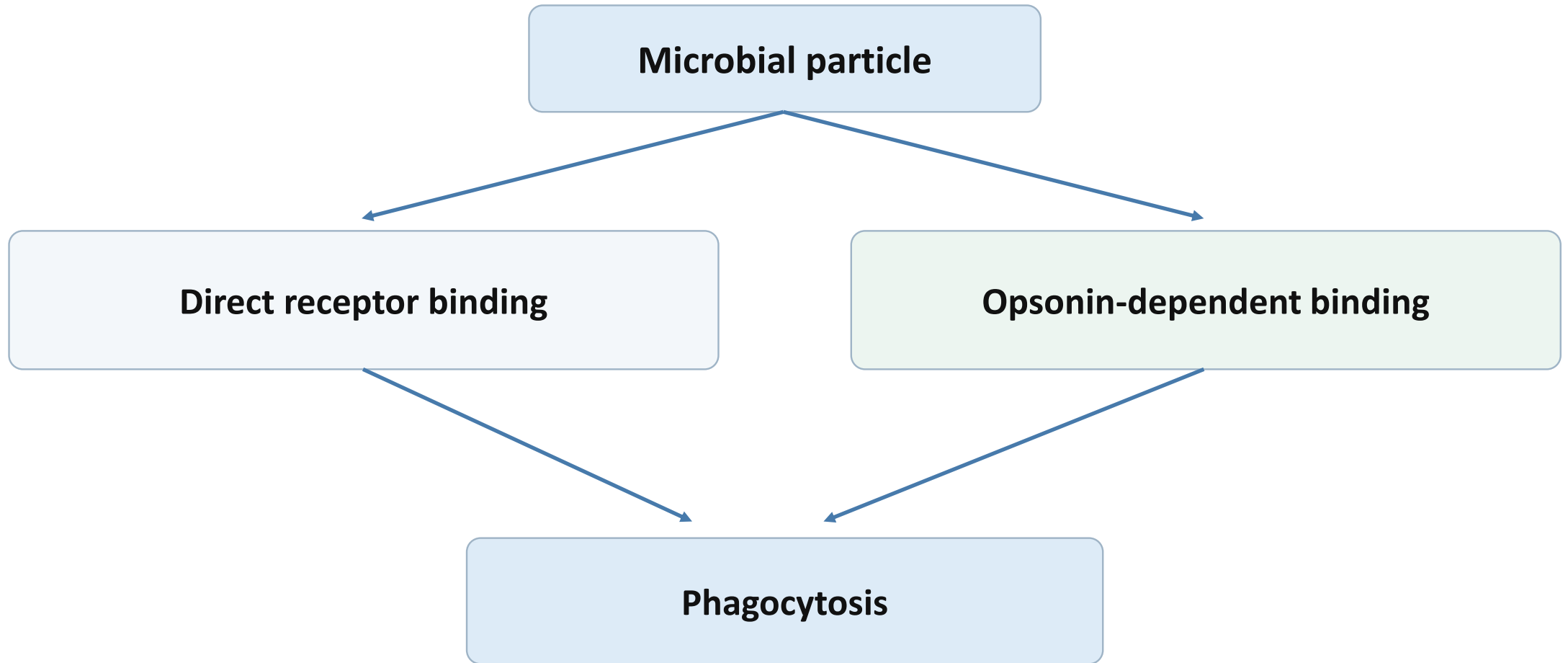
- Many terminal sugars are modified.
- Sialic acid or N-acetylgalactosamine helps distinguish host cells.

Opsonins amplify phagocytic efficiency

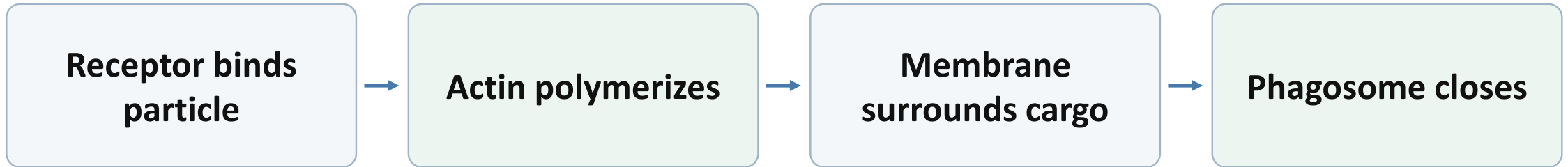


- Main opsonins: IgG; Complement-derived C3b; Plasma lectins.
- This provides an indirect recognition pathway alongside direct binding.

Recognition pathways converge on engulfment



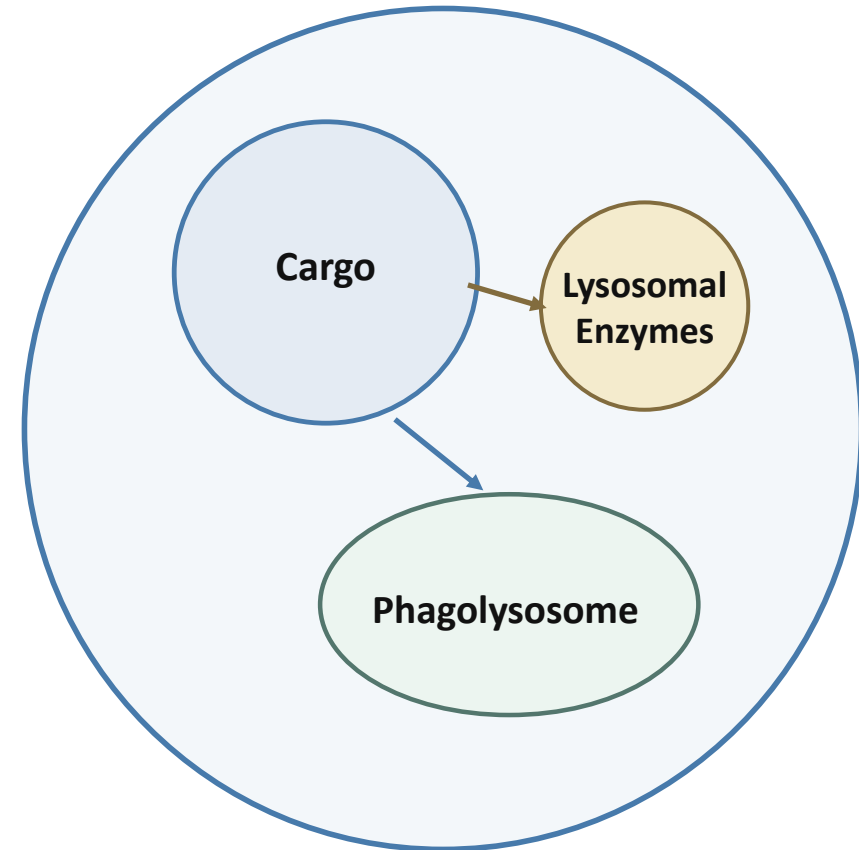
Engulfment is an actin-dependent event



- Actin-driven cytoskeletal remodeling explains the membrane movement.
- The microbe remains enclosed in a membrane-bound compartment.

Fusion forms the microbicidal compartment

- Lysosomal granules fuse with the phagosome.
- Hydrolases and microbicidal substances enter the phagolysosome.
- Delivery into this compartment concentrates destructive activity.



Compartmentalization protects the phagocyte

Contained killing

- Toxic products act near ingested cargo.
- Cytoplasm and nucleus are spatially shielded.

Extracellular escape

- Oxidants and proteases can reach healthy tissue.
- The same effectors now cause collateral injury.

Three major intracellular effector systems

Reactive oxygen species

Produced rapidly after phagocyte oxidase assembly.

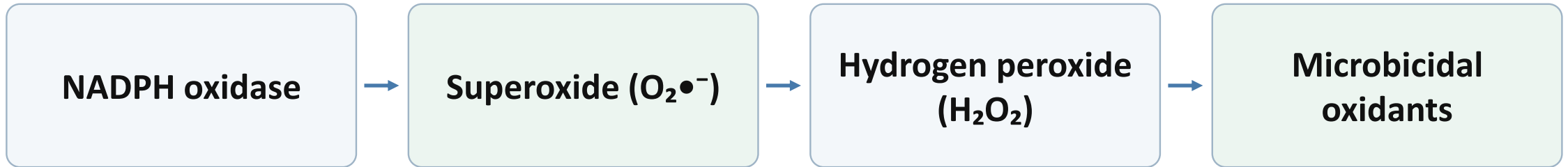
Reactive nitrogen species

Generated via inducible nitric oxide synthase.

Lysosomal contents

Hydrolases, proteases and antimicrobial proteins.

Reactive oxygen species: Pathway overview



- MPO can use hydrogen peroxide to make hypochlorite.
- Hydroxyl radicals and related oxidants also damage cell components.

Phagocyte oxidase is inactive as a dispersed complex

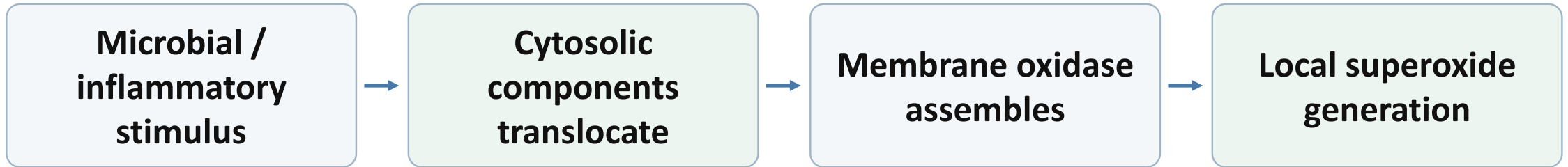
Resting neutrophil

- Enzyme components reside in different locations.
- Some components are membrane associated.
- Others are distributed in the cytoplasm.

Functional requirement

- Stimulus-dependent assembly at a membrane.
- Assembly brings the oxidase components together.

Activation assembles NADPH oxidase on the phagosome



- ROS synthesis is concentrated at the site of ingested cargo.
- The complex comprises multiple protein components.

Respiratory burst is rapid oxygen consumption

Trigger

A stimulus activates the phagocyte oxidase system.

Biochemical event

Oxygen is used to form superoxide anion.

Functional purpose

Generate antimicrobial oxidants in the phagosome.

NADPH oxidase initiates oxygen reduction

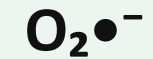


Reduced electron donor



NADPH oxidase

Electron transfer



Superoxide anion

The important first product is superoxide, not hypochlorite.

Superoxide produces hydrogen peroxide



Primary oxidant



Dismutation

Spontaneous conversion

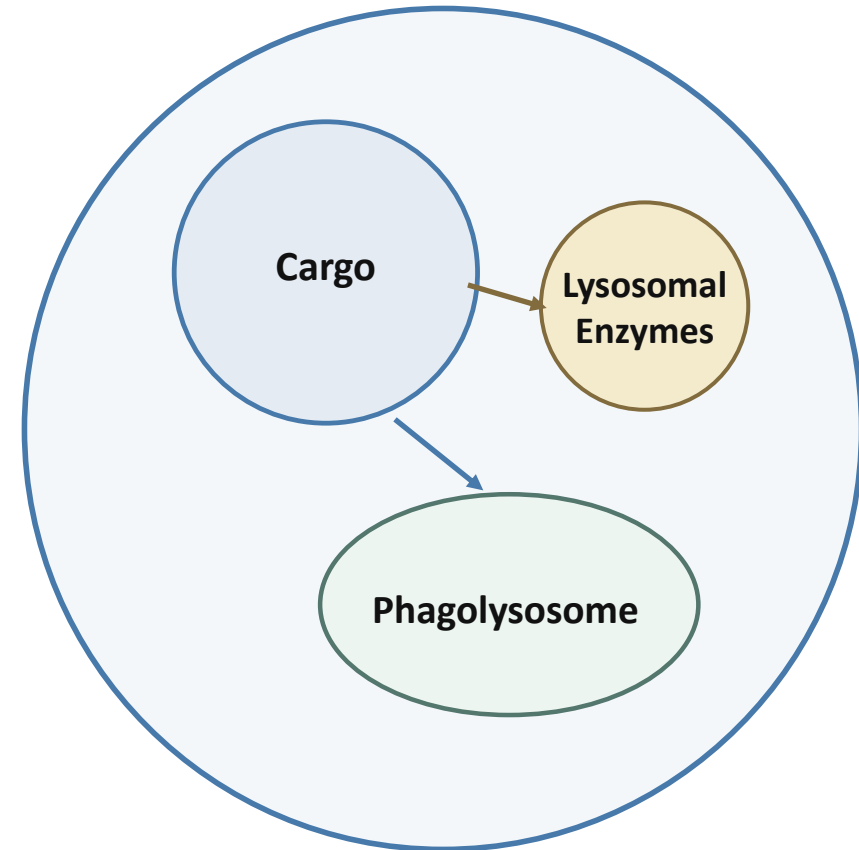


Substrate for further oxidants

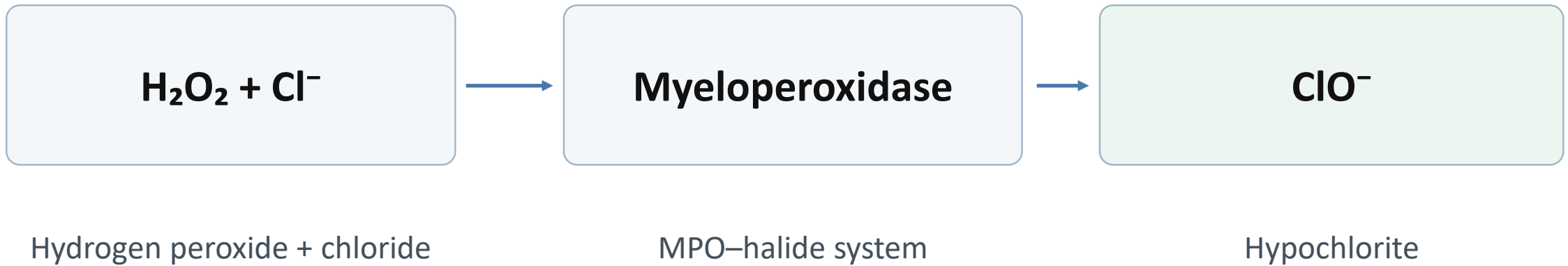
Hydrogen peroxide is an intermediate in the stronger neutrophil killing system.

Myeloperoxidase is a primary-granule enzyme

- Azurophilic (primary) granules are rich in MPO.
- Granule fusion supplies MPO to the phagolysosome.
- MPO uses H_2O_2 and a halide to generate a stronger antimicrobial oxidant.

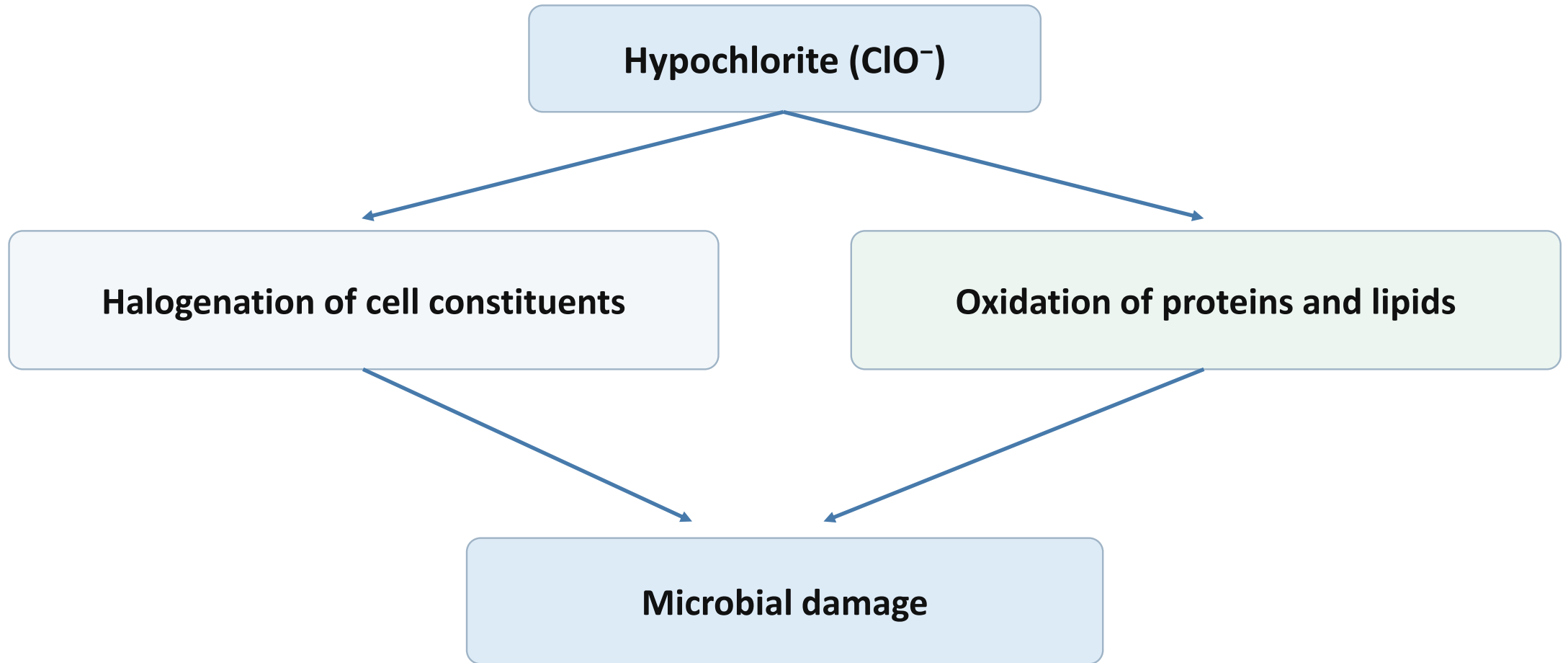


MPO generates hypochlorite

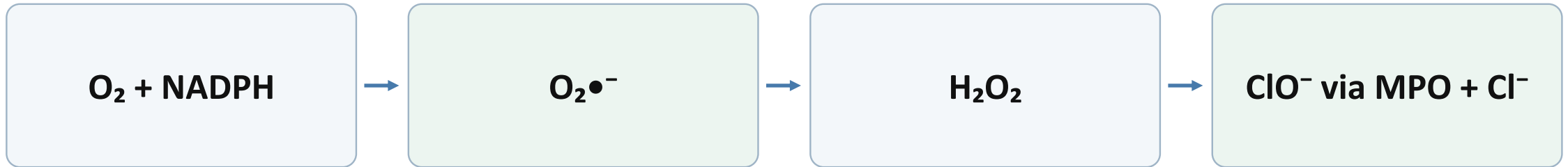


The H_2O_2 –MPO–halide pathway is the most potent bactericidal system of neutrophils.

Hypochlorite injures microbial molecules

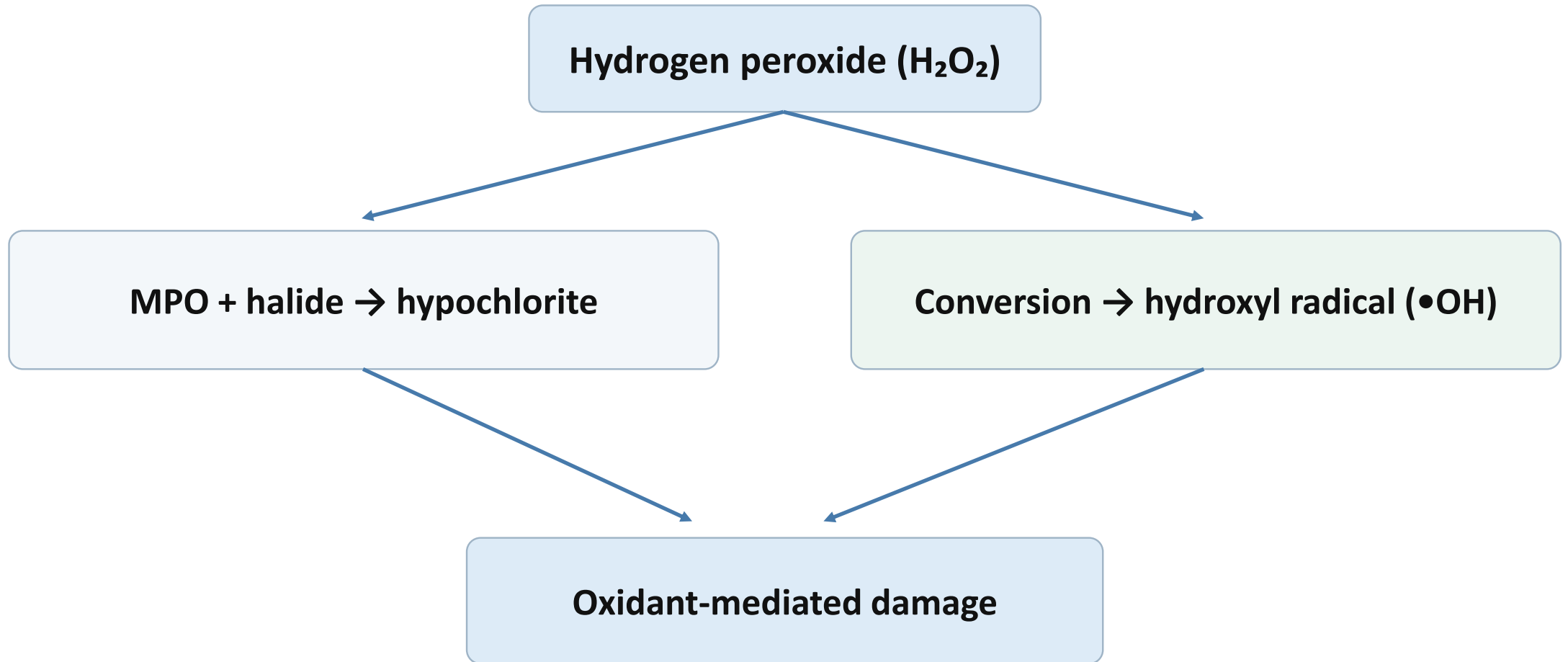


The neutrophil ROS cascade, integrated



- Several oxidants and enzymes act together rather than as one isolated reaction.
- Main site: Phagolysosome after membrane oxidase assembly.

Hydroxyl radical provides another toxic route



Oxidants target multiple microbial macromolecules

Lipids

Oxidative disruption of cellular membranes.

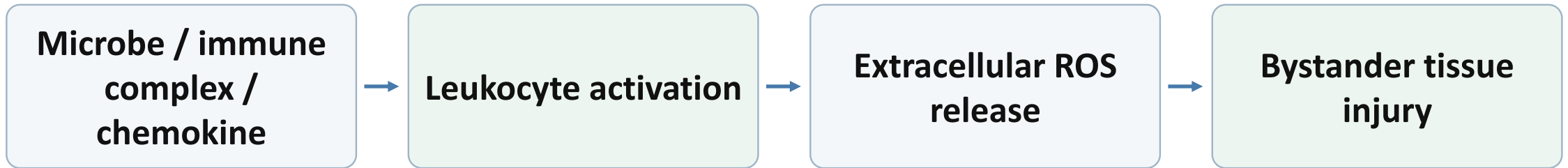
Proteins

Chemical modification impairs structure and function.

Nucleic acids

Reactive species chemically damage genetic material.

ROS are not restricted to the phagolysosome



- Extracellular release converts defense into a source of inflammation-associated injury.
- Tissue damage depends on whether ROS reach host structures.

Antioxidants buffer oxidant injury

Cellular enzymes

- Superoxide dismutase
- Catalase
- Glutathione peroxidase

Tissue and plasma protection

- Ceruloplasmin
- Iron-free transferrin fraction
- Multiple protective mechanisms operate together.

Superoxide dismutase limits the superoxide burden

Substrate

Superoxide anion is a reactive first product.

Antioxidant

Superoxide dismutase participates in oxidant handling.

Balance

Defense systems reduce exposure to harmful radicals.

Catalase and glutathione peroxidase neutralize H_2O_2

Catalase

- Detoxifies hydrogen peroxide.
- Protects cells from oxidant accumulation.

Glutathione peroxidase

- Also removes hydrogen peroxide.
- Provides an additional protective route.

Plasma antioxidants add extracellular protection

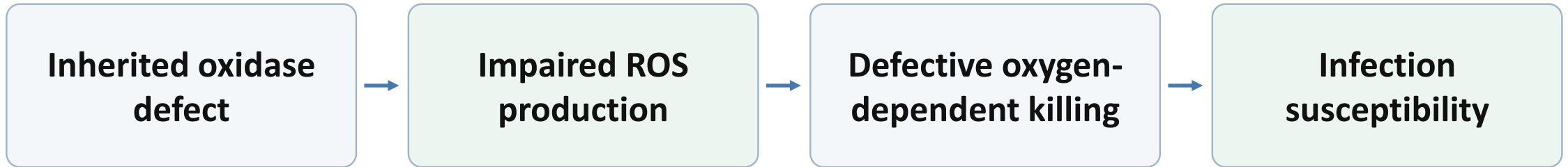
Ceruloplasmin

- Copper-containing plasma protein.
- Included among protective antioxidant mechanisms.

Transferrin

- Iron-free transferrin fraction is protective.
- Represents another defense in tissue fluids.

Chronic granulomatous disease: Oxidase deficiency



- This is a phagocyte oxidase immunodeficiency.
- The topic is developed further in the immunodeficiency chapter.

MPO deficiency demonstrates microbicidal redundancy

MPO deficiency

- One strong oxidant-generating route is deficient.
- Minimal increase in infection susceptibility.

NADPH oxidase deficiency

- Upstream ROS generation is impaired.
- Associated with chronic granulomatous disease.

Redundant killing explains a clinical paradox

Observation

MPO–halide chemistry is highly bactericidal.

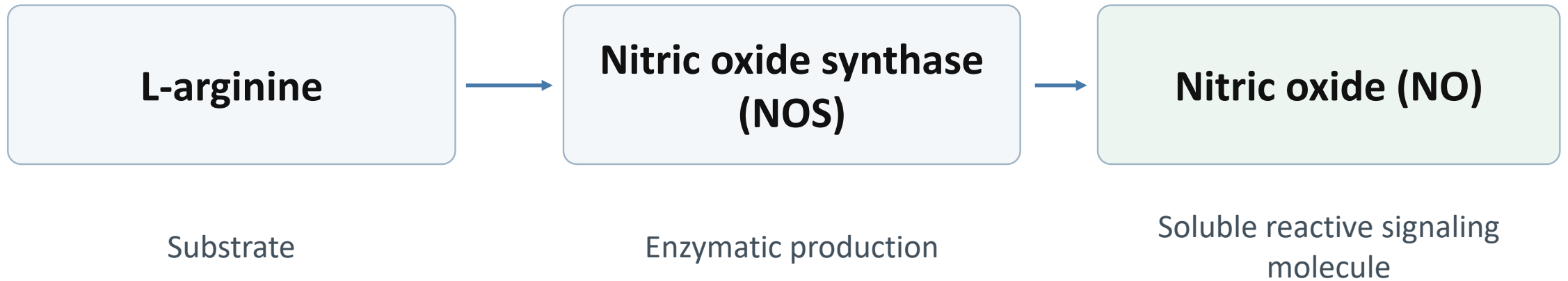
Observation

Inherited MPO deficiency often has little infection impact.

Inference from source

Other leukocyte effectors compensate for an isolated loss.

Reactive nitrogen species begin with nitric oxide

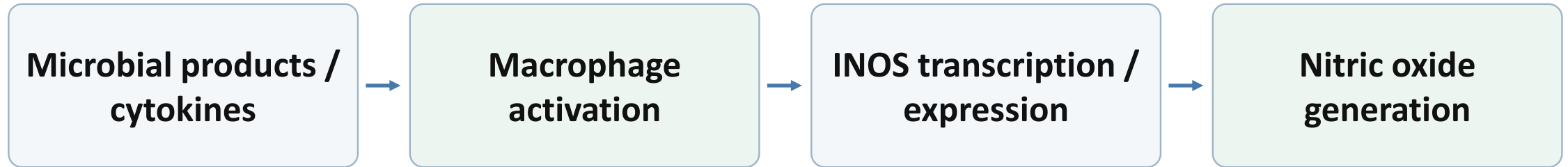


The inducible NOS isoform participates in microbial killing.

Three nitric oxide synthases have distinct roles

Isoform	Expression / function
eNOS	Constitutive, low-level NO; vascular tone
nNOS	Constitutive, low-level NO; neurotransmission
iNOS	Induced in activated phagocytes; microbial killing

Inducible NOS responds to macrophage activation



- Interferon- γ is one cited induction signal.
- Neutrophils can contribute, but macrophage iNOS is emphasized.

Macrophages: Nitric oxide as an antimicrobial effector

Activation input

- Microbial products
- Cytokines including interferon- γ

Effector output

- Induced nitric oxide synthesis
- Reactive nitrogen-derived antimicrobial damage

NO and superoxide form peroxynitrite



Nitric oxide + superoxide



Chemical reaction

Reactive species combine



Peroxynitrite

Peroxynitrite provides a powerful nitrogen-derived microbicidal oxidant.

ROS and RNS share major molecular targets

Oxygen-derived species

- Oxidize lipids, proteins and nucleic acids.
- Include superoxide-derived oxidants.

Nitrogen-derived species

- Damage the same classes of targets.
- Include NO-derived peroxynitrite.

Experimental evidence illustrates overlap

Single pathway missing

Mice lacking oxidase OR iNOS
have only mild infection
susceptibility.

Both pathways missing

Double-knockout mice develop
severe disseminated infection.

Interpretation

Cooperating ROS and RNS
pathways provide functional
backup.

NO has an additional vascular action

Microbicidal setting

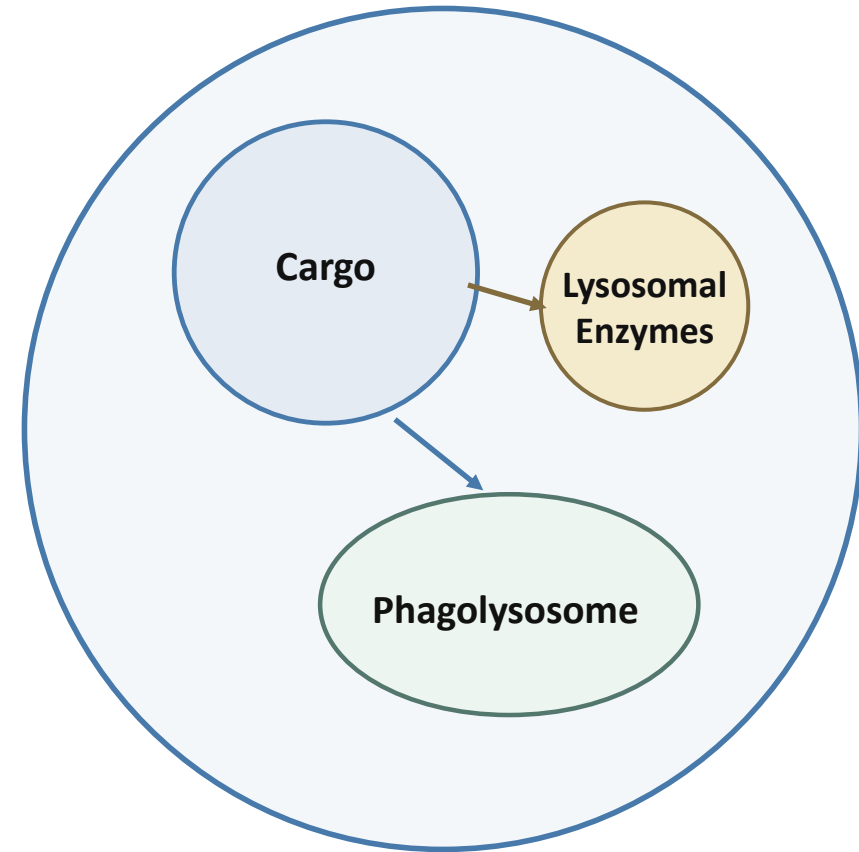
- INOS-derived NO acts in activated macrophages.
- Can yield peroxynitrite with superoxide.

Vascular setting

- NO relaxes vascular smooth muscle.
- Its precise contribution to acute inflammatory vascular changes is uncertain.

Granules organize nonoxidative killing

- Lysosomal granules store enzymes and antimicrobial proteins.
- Fusion delivers these effectors into the ingested cargo compartment.
- Discharge outside the cell can damage tissue.



Neutrophils carry two major granule populations

Primary / azurophilic

- Larger granules.
- MPO; Defensins; Lysozyme; Acid hydrolases.
- Neutral proteases including elastase.

Secondary / specific

- Smaller granules.
- Lysozyme; Collagenase; Gelatinase.
- Lactoferrin and additional enzymes.

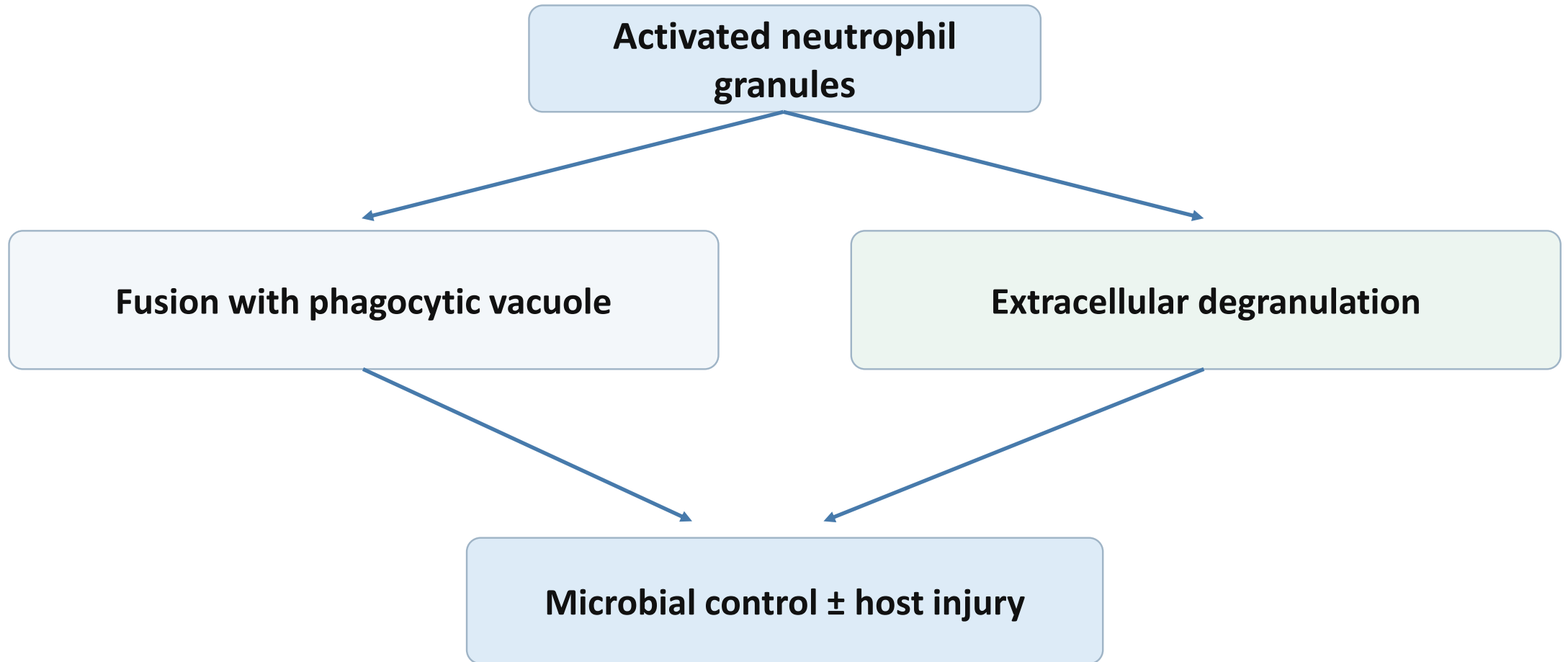
Primary granules combine oxidants and proteases

Primary granule constituent	Principal relevance
Myeloperoxidase	H ₂ O ₂ –halide oxidant generation
Defensins and lysozyme	Direct antimicrobial effects
Acid hydrolases	Digestion of phagocytosed material
Elastase / cathepsin G / proteinase 3	Proteolytic activity

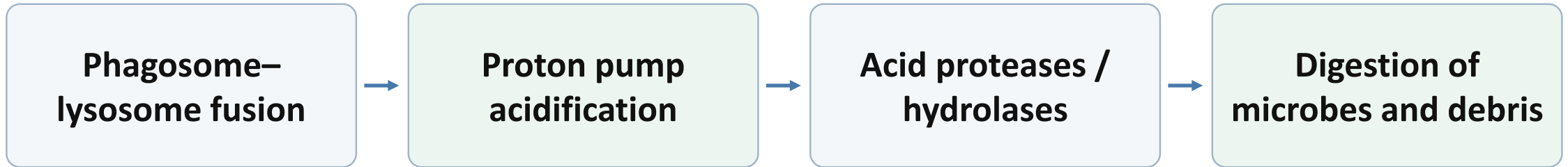
Secondary granules broaden enzymatic activity

Secondary granule constituent	Functional class
Lysozyme	Antibacterial enzyme
Collagenase and gelatinase	Matrix-degrading enzymes
Lactoferrin	Iron-binding antimicrobial protein
Histaminase / alkaline phosphatase	Other granule enzymes

Granules may fuse internally or release externally



Acidified phagolysosomes favor digestion



- Compartment acidification creates an enzymatic environment for digestion.
- This route is mechanistically distinct from oxidant-mediated killing.

Neutral proteases have broad extracellular targets

Extracellular matrix

Collagen; Basement membrane;
Elastin; Cartilage.

Protein deposits

Fibrin is susceptible to
degradation.

Inflammatory mediators

Proteases can process
complement and kinin
pathways.

Elastase can protect and injure

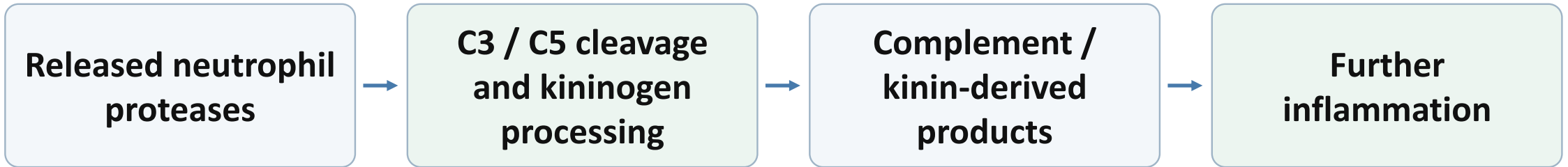
Host defense

- Neutrophil elastase degrades bacterial virulence factors.
- Contributes to antimicrobial activity.

Tissue damage

- Uncontrolled elastase degrades elastic support structures.
- Damage depends on antiprotease protection.

Proteases can amplify mediator cascades



- Protein degradation is not limited to structural damage.
- The source describes mediator-generating effects of neutral proteases.

Macrophages also contain degradative enzymes

Macrophage enzymes

- Acid hydrolases and phospholipase
- Collagenase and elastase
- Plasminogen activator

Functional implication

- Digestion of engulfed debris
- Potential connective-tissue remodeling
- Potential tissue injury if released

Antimicrobial granule peptides act directly

Defensins

- Cationic, arginine-rich granule peptides.
- Direct toxicity to microorganisms.

Cathelicidins

- Antimicrobial proteins of neutrophils and other cells.
- Additional antimicrobial defense.

Lysozyme and lactoferrin act differently

Lysozyme

- Hydrolyzes the muramic acid–N-acetylglucosamine bond.
- Acts on bacterial cell-wall components.

Lactoferrin

- Iron-binding protein in specific granules.
- Contributes to antimicrobial granule functions.

Eosinophil major basic protein: A distinct example

Cell context

Major basic protein is a cationic eosinophil protein.

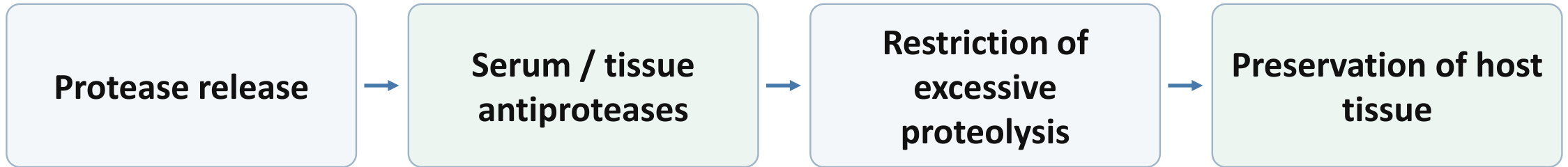
Antibacterial action

Its bactericidal activity is limited.

Parasite defense

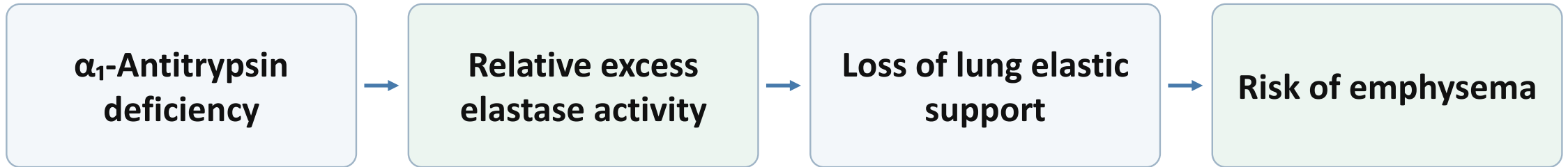
It is cytotoxic to many helminthic parasites.

Antiproteases restrain leukocyte-mediated injury



- Antiprotease control is essential because enzymes cannot intrinsically select host versus microbe.
- α_1 -Antitrypsin and α_2 -macroglobulin are named protective proteins.

α_1 -Antitrypsin restrains neutrophil elastase



- This is a mechanism linking inflammatory proteolysis to organ pathology.
- α_1 -antitrypsin as the major neutrophil elastase inhibitor.

α_2 -Macroglobulin provides another antiprotease layer

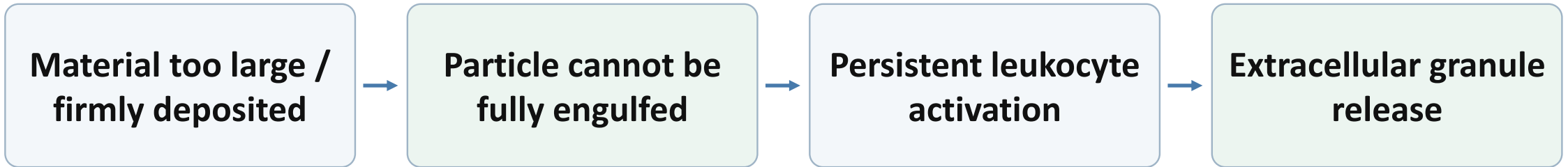
Protease challenge

- Activated leukocytes release multiple proteases.
- Serum and tissue fluids require counter-regulation.

Protective response

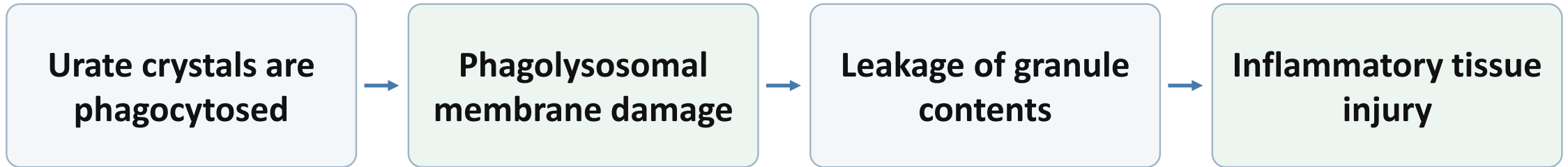
- α_2 -Macroglobulin is a circulating antiprotease.
- It supplements other inhibitory proteins.

Frustrated phagocytosis releases toxic contents



- Example in source: Immune complexes deposited on a broad surface.
- Extracellular enzymes can injure host tissue nearby.

Ingested crystals can breach the compartment



- The protective phagolysosomal barrier may fail after particular challenges.
- This mechanism connects particle uptake to collateral damage.

Leukocytes injure tissues through their defense machinery

Oxidants

Extracellular ROS modify host molecules.

Reactive nitrogen species

RNS may contribute to cellular injury.

Proteases

Matrix and membrane constituents are degraded.

Three contexts for collateral leukocyte injury

Persistent infection

Prolonged responses damage adjacent tissues.

Autoimmunity

Inflammatory effectors are directed toward self tissues.

Allergy

Excess responses target usually innocuous stimuli.

Damage may arise without a defect in phagocytosis

Effective containment

- Microbes are engulfed and lysed intracellularly.
- Microbicidal substances remain largely compartmentalized.

Mislocalized effectors

- ROS and granule contents reach extracellular structures.
- Inflammation itself becomes injurious.

Mechanism comparison: Deficiency versus excess

Disturbance	Mechanism	Pathologic implication
Oxidase deficiency	Reduced antimicrobial ROS	Susceptibility to infection (CGD)
MPO deficiency	Less hypochlorite production	Often mild impact because of redundancy
Antiprotease deficiency	Unopposed elastase	Lung elastic-fiber destruction
Extracellular release	Oxidants / enzymes hit host tissue	Inflammation-associated injury

Clinical reasoning: Defective upstream oxidant formation

A patient is known to have an inherited defect in the phagocyte oxidase complex.

Which part of microbial killing is directly compromised?

Interpretation

Production of superoxide during the respiratory burst is impaired, reducing downstream ROS generation.

Clinical reasoning: Strong MPO biochemistry, mild deficiency

A patient has isolated inherited myeloperoxidase deficiency with little infection susceptibility.

How can this occur despite the potency of the MPO–halide system?

Interpretation

Leukocyte killing is redundant; Other ROS, RNS and lysosomal mechanisms can partly compensate.

Clinical reasoning: Emphysema and protease balance

A patient has α_1 -antitrypsin deficiency and loss of lung elastic support.

Which leukocyte effector explains this connection?

Interpretation

Neutrophil elastase is insufficiently inhibited, increasing elastin and matrix destruction.

Clinical reasoning: Broad extracellular immune deposits

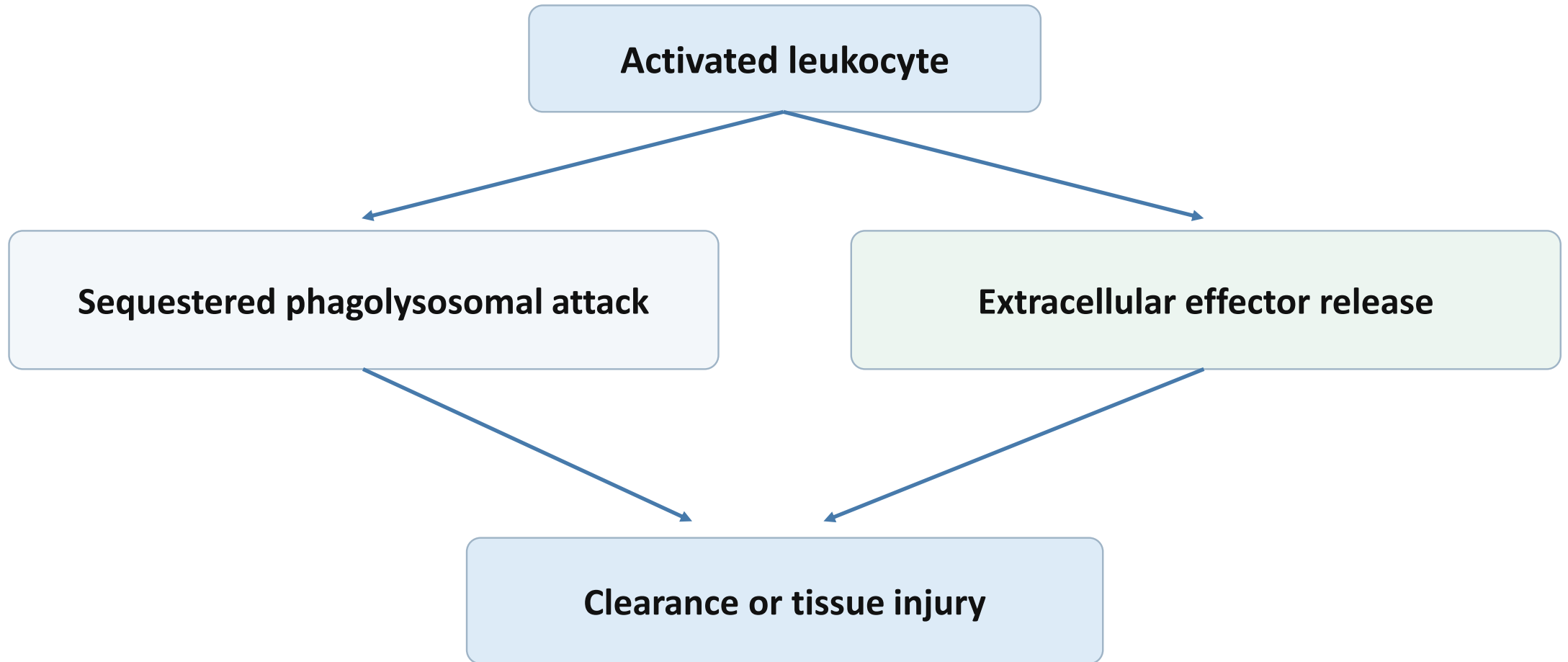
Immune complexes are deposited across a broad tissue surface that a neutrophil cannot surround.

What produces injury even without complete particle ingestion?

Interpretation

Frustrated phagocytosis leads to granule release and extracellular proteolytic injury.

Integrated sequence: Defense and collateral damage



Five mechanistic conclusions

- NADPH oxidase produces superoxide; MPO transforms H_2O_2 into hypochlorite.
- Induced nitric oxide can generate peroxynitrite with superoxide.
- Acidified phagolysosomes deliver proteolytic and antimicrobial proteins.
- Antioxidants and antiproteases limit self-inflicted damage.
- Disrupted containment or defective killing explains important disease links.

Primary textbook reference

- Robbins, Cotran & Kumar. Pathologic Basis of Disease. 11th edition (2026).
- Chapter 3: Inflammation and Repair — phagocytosis and intracellular destruction of microbes and debris.
- Relevant textbook material: printed pp. 72–76; diagrams discussed conceptually without reproducing textbook figures.

All original diagrams in this presentation are editable teaching schematics; they are not clinical or histologic specimens.