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Inflammation: Purposes, Mechanisms and Development

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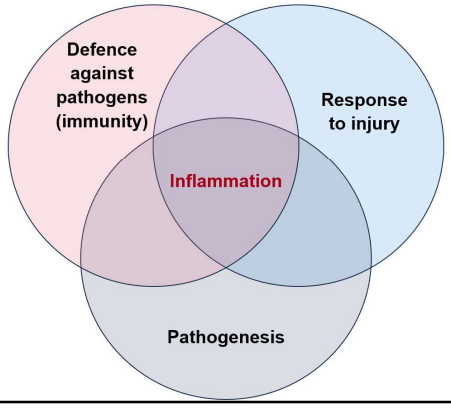
The inflammatory response can be discussed in different-contexts



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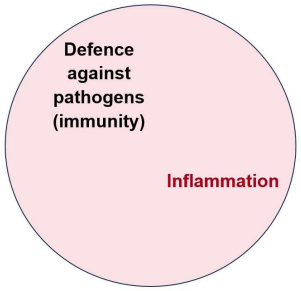

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What is the "function" of the inflammatory response?

1 Role of inflammation in host defense from pathogens as its main purpose

2 Non-infective (sterile) inflammation and its main purposes

4

Inflammation as an aspect of innate immunity

5

Types of mechanism that let us survive an infection

Pathogen control

Decreases the number of pathogens (killing or growth inhibition)

Damage control

Protects from the damage induced by the infection

Immunity

Innate | Adaptive

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Innate immunity

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Innate immunity is the first cell-mediated response of the immune system aimed at reducing the number of pathogens

Fast

Activated immediately (minutes) after infection

Unlike adaptive immune response (days needed)

Germline encoded

Innate immune receptors that recognize pathogens:

Are germline encoded

Do not require previous exposure to the pathogen for recognition

The acute inflammatory response to an infection - overview

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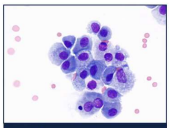
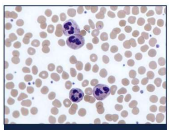
1. Cells recognize pathogens through pattern recognition receptors (PRR)
2. PRR activate transcription factors leading to expression of inflammatory genes: cytokines, chemokines and other inflammatory mediators
3. Cytokines induce systemic effects (e.g. fever and acute-phase response)
4. Circulating leukocytes (neutrophils and monocytes) extravasate from the blood to the tissue at the site of infection where they eliminate the pathogens
5. When pathogens are eliminated, inflammation resolves with minimal tissue damage



Macrophages



Neutrophils

Pattern recognition receptors (PRRs)

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Receptors of adaptive immunity	Receptors of innate immunity
■ B-cell receptors and T-cell receptors	■ Do not recognize specific antigens
■ Huge diversity (trillions of possible conformations)	■ Recognize molecular patterns common to many pathogens (pathogen-associated molecular patterns - PAMP)
■ Recognize specific antigens, such as a portion of a foreign protein (epitope)	■ Less specific but able to distinguish potentially pathogenic microbes from non-pathogenic ones

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PRRs recognize PAMP

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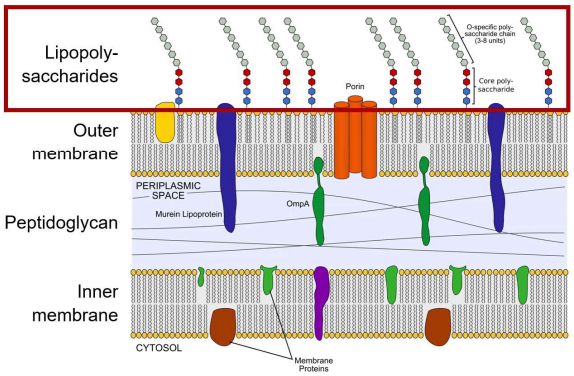
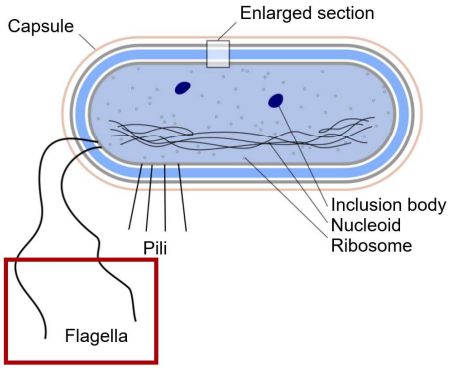
- Unlike receptors of adaptive immunity, receptors of innate immunity cannot learn and adapt like those of adaptive immunity
- Therefore must recognize **highly conserved structures** (cell wall, nucleic acids)
- Examples of PAMP are **lipopolysaccharides** (LPS) or **flagellin** (component of the flagella) of gram-negative bacteria

PRRs recognize PAMP



PRRs recognize PAMP



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PRRs recognize PAMP

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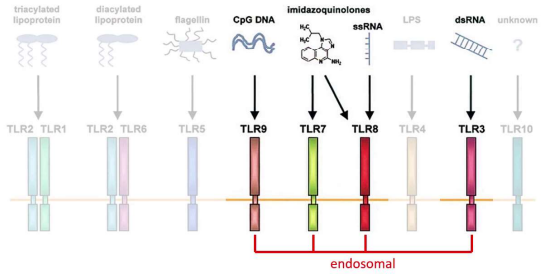
A major class of PRRs is that of the **TLRs: Toll-like receptors**

- On the cell membrane or, intracellularly, on the endosomal membrane
- Recognize different extracellular or intracellular PAMPs, including viral RNAs



PRRs recognize PAMP

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PRRs present in the cytoplasm (to recognize viral RNAs or intracellular bacterial products):

RLRs: Retinoic acid-inducible gene-I (RIG-I)-like receptors

- RIG-I, MDA5 (viruses)

NLRs: Nucleotide-binding oligomerization domain (NOD)-like receptors

- NOD1, NOD2, NALP1, IPAF, NAIP5 (bacteria)
- NALP3 (bacteria, viruses)
- CLR (fungi)

PRRs trigger signaling cascades

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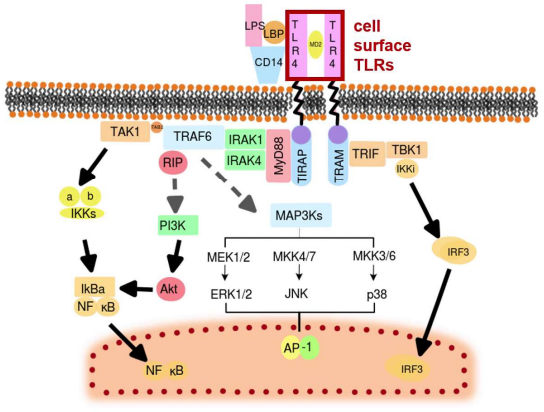

HSTalks

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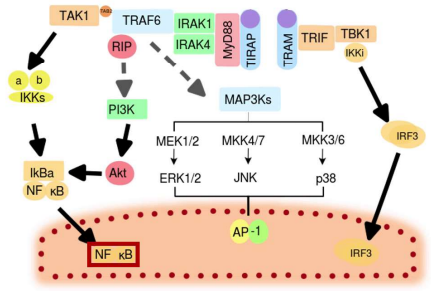
PRRs trigger signaling cascades

- Activation of **transcription factors** (particularly NF-kB)
- Induction of **inflammatory cytokines** (e.g. IL-1, IL-6, TNF)
- Induction of **enzymes** producing prostaglandins or nitric oxide



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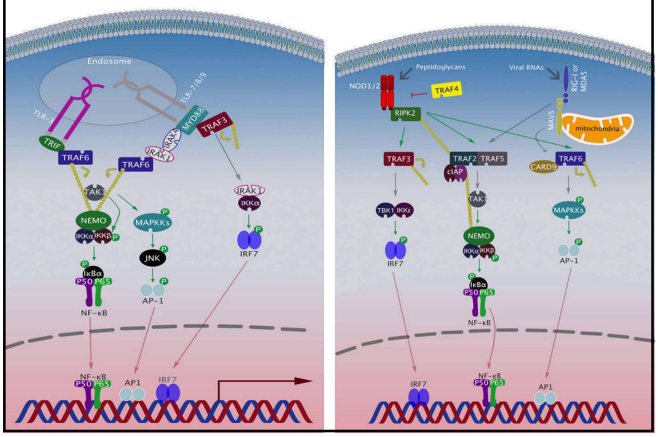
Endosomal and cytoplasmic TLRs



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NF-kB target genes involved in inflammation

	Examples	Roles
Inflammatory cytokines	IL-1, IL-6, IL-12, TNF	Various
Growth factors	G- M- and GM-CSF	Increase leukocyte differentiation
Chemokines	IL-8, MIP1, MCP, GRO	Leukocyte migration/activation
Enzymes	iNOS	Produces nitric oxide
	COX-2, PLA2	Produce prostaglandins and leukotrienes
Adhesion molecules	E-selectin, ICAM-1, VCAM-1	Leukocyte extravasation

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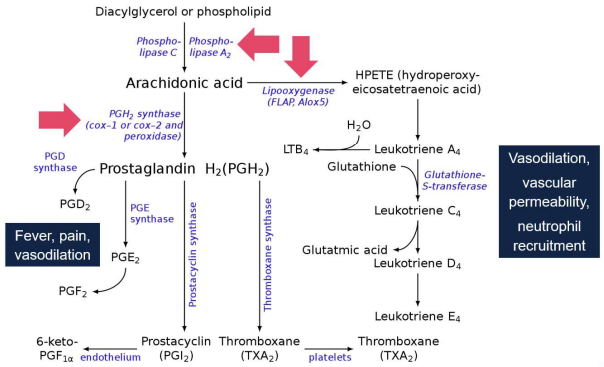
Biosynthesis of eicosanoids from arachidonic acid



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Leukocyte extravasation and the hallmarks of inflammation

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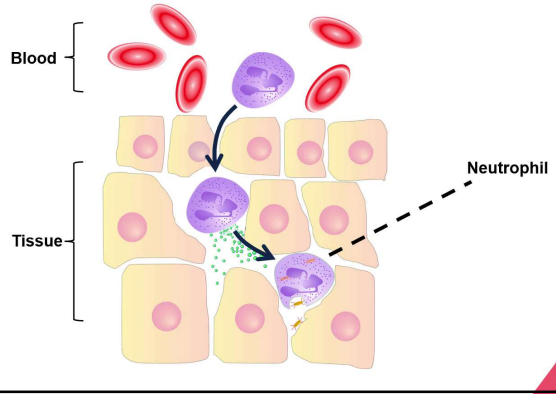
Early step in the inflammatory response:

Extravasation of leukocytes from the blood to the site of infection/inflammation



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Key (protein) players in the process of tissue infiltration of inflammatory cells

Inflammatory cytokines (e.g. TNF, IL-1, IL-6)	Chemokines (e.g. IL-8/CXCL8 for neutrophils; MCP1 /CCL2 for monocytes/ macrophages) & other chemotactic factors (e.g. C5a, fMLP)	Adhesion molecules
Inflammatory cytokines activate the endothelium	Chemokines and other chemotactic factors induce migration	Adhesion molecules promote adhesion to the vascular endothelium and extravasation

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Main steps in the infiltration of inflammatory cells

1. Vasodilation and increased vascular permeability (cytokines, kinins)
2. Leukocytes rolling and adhesion to the vascular endothelium
3. Normal endothelium does not bind circulating leukocytes; needs activation by cytokines
4. Migration through the endothelium and the vessel wall
5. Migration to tissues following a chemotactic stimulus

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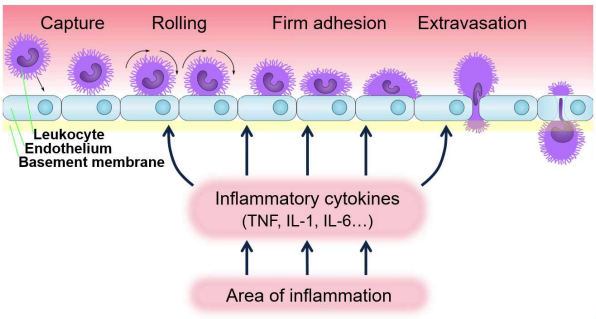
Capture, rolling and adhesion depend on specific adhesion molecules



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Capture, rolling and adhesion depend on specific adhesion molecules

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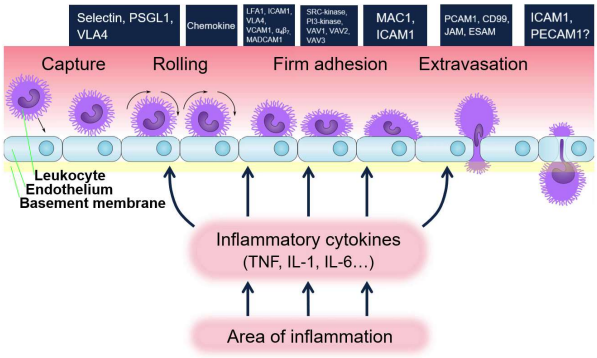
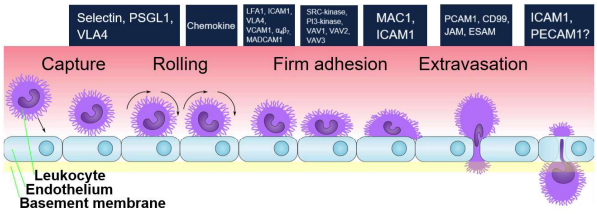


Capture, rolling and adhesion depend on specific adhesion molecules



- Expression of **selectins** and their ligands is induced by inflammatory cytokines
- Preformed P selectin is rapidly redistributed from intracellular storage (Weibel-Palade bodies) to the endothelial cell surface
- Firm adhesion is due to **integrins** on leukocytes being activated to a high affinity state by chemokines
- Leukocytes produce **collagenase** to disrupt the basement membrane

Summary

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1. Leukocytes are recruited to the site of infection by chemotactic factors: bacterial molecules such as fMLP; complement products (e.g. C5a); chemokines (e.g. IL-8)
2. Requires extravasation of circulating leukocytes (neutrophils & monocytes)
3. Extravasation is associated to increased vascular permeability (e.g. by TNF or inflammatory eicosanoids such as prostaglandins and leukotrienes)
4. Circulating leucocytes arrest because they bind the vascular endothelium via adhesion molecules, mainly endothelial selectin
5. Binding is strengthened due to the binding of leukocyte integrins (activated by chemokines) to their endothelial ligands (e.g. ICAM-1)
6. Transendothelial passage involves other adhesion molecules (e.g. PECAM-1) and collagenase to disrupt the basement membrane

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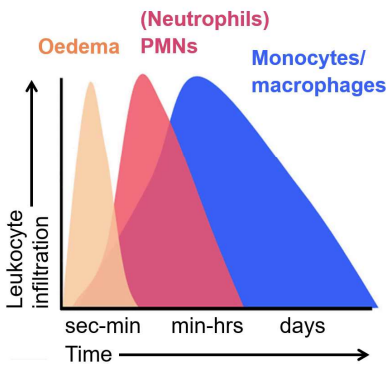
Kinetics of acute inflammation



- Neutrophils are the first to infiltrate the site of inflammation
- Monocytes follow (that, in the tissue, will differentiate to macrophages)
- Neutrophils in tissues are short lived

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Effectors of inflammation

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Hallmarks of inflammation

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Hallmarks of inflammation

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- Calor (heat) — Vasodilation
- Rubor (redness) — Vasodilation
- Tumor (swelling) — Increased vascular permeability
- Dolor (pain) — Nociceptor stimulation

- The first three can be explained by the action of inflammatory cytokines on the vessel wall
- Pain is probably mainly due to bradykinin but also due to tumor necrosis factor

Effector mechanisms of infiltrated leukocytes in the defense against pathogens

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1. **Phagocytosis** (neutrophils, monocytes/macrophages)
2. **Antimicrobial peptides** (neutrophils)
3. **Lytic enzymes** (neutrophils, monocytes/macrophages)
4. **Reactive oxygen and nitrogen species** (neutrophils, monocytes/macrophages)
5. **Acidification** (neutrophils, monocytes/macrophages)
6. **Inflammatory mediators** (monocytes/macrophages)
7. **Antigen presentation** (monocytes/macrophages)
8. **Cytokines** (monocytes/macrophages)

Inflammatory cell infiltrate

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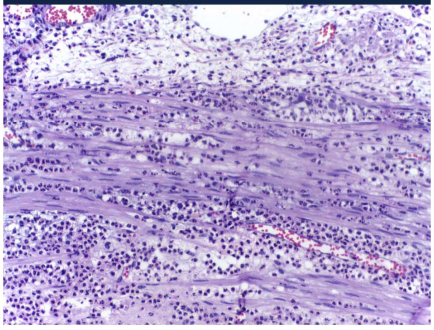
Nowadays, the inflammatory cell infiltrate is commonly considered the hallmark of inflammation



Source: Department of Pathology, Calicut Medical College

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Systemic effects of inflammation: fever and the acute-phase response

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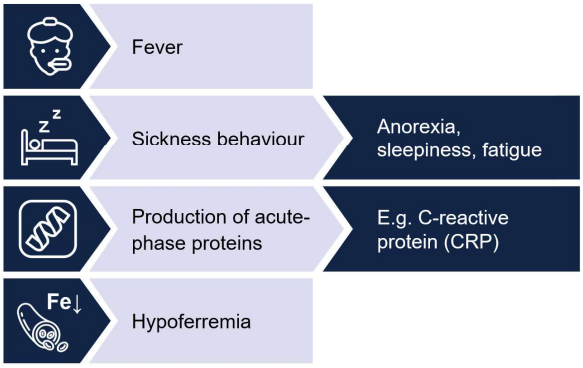
Infections can cause several systemic effects



Infections can cause several systemic effects

- These are largely due to the **pleiotropic activity** of cytokines outside the immune system
- Pleiotropic: cytokines can act on many different types of cells, both within and outside of the immune system

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Infections can cause several systemic effects

Fever	(IL-1 → brain) → stimulates phagocytosis and killing
Sickness behaviour	(brain) → energy conservation to fight infection
Production of acute-phase proteins	(IL-6 → liver) → CRP opsonization
Fe↓ Hypoferremia	(IL-6 → liver → hepcidin) → iron is required by microorganisms

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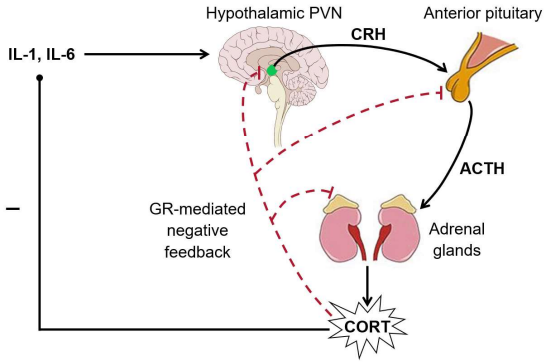
Inflammatory cytokines activate the hypothalamus-pituitary-adrenal axis

Inflammatory cytokines activate the hypothalamus-pituitary-adrenal axis (HPAA) resulting in an increase in blood corticosteroids, potent anti-inflammatory molecules

Inflammatory cytokines activate the hypothalamus-pituitary-adrenal axis



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Inflammation as a response to injury and the concept of DAMP

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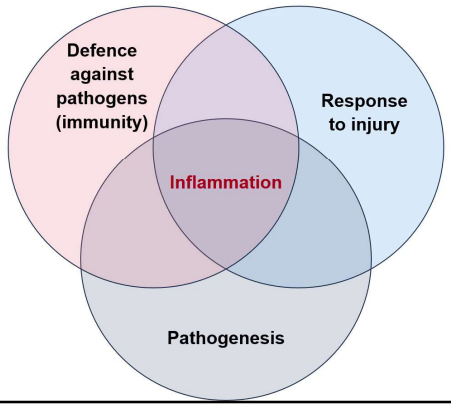
The inflammatory response can be discussed in different-contexts



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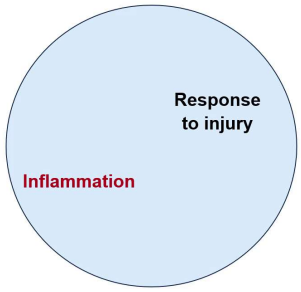

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Inflammation in the absence of infection (sterile inflammation)

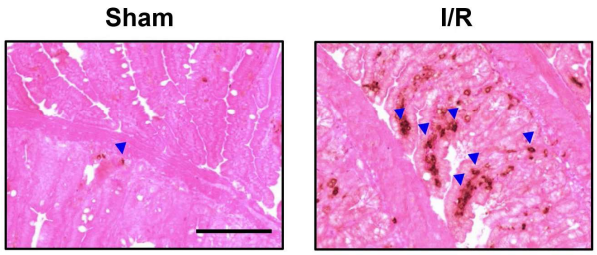
Ischemic injury

Tissue damage (e.g. wounds)

Chemicals & foreign bodies (e.g. urate or silica crystals)

Atherosclerosis

Inflammation in the absence of infection (sterile inflammation)



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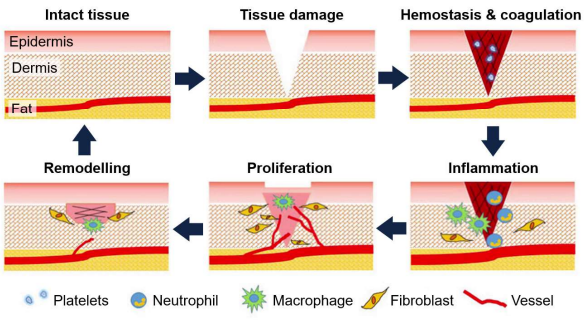
Inflammation after tissue damage

Inflammation after tissue damage is important for the elimination of dead cells, debris or microorganisms and activation of the fibrotic response



Morbidelli L. *et al.*, *Front Bioeng Biotechnol.* 2021; **9**:720091

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Non-microbial agents can induce inflammation

Infections

Pathogen-Associated Molecular Patterns (PAMPs) activate various PRRs

Damage/stress

Damage-Associated Molecular Patterns (DAMPs) released in response to injury activate various PRRs

DAMPs can include crystals (e.g. urate, silica) or molecules normally present only inside the cells (e.g. ATP, nuclear proteins)

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Main damage-associated molecular patterns

DAMP	Receptor
Nuclear proteins (histones, HMGB1)	TLR2,4 and 9; RAGE
DNA	TLR9, NALP3
ATP	P2X7/NALP3
Heat-shock proteins	TLR2, 4
S100	TLR2, 4 and RAGE
ECM components (fibrinogen, hialuronate, tenascin, heparans)	TLR2,4; NALP3
Urate crystals, cholesterol, asbestos	NALP3
Oxidative stress	NALP3

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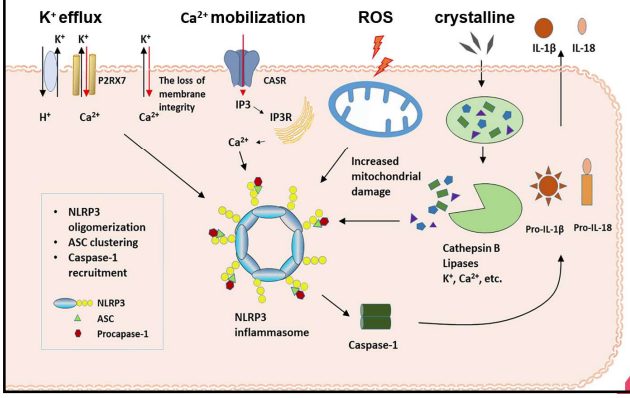
The NALP3 inflammasome is implicated in the response to DAMPs



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Resolution of inflammation

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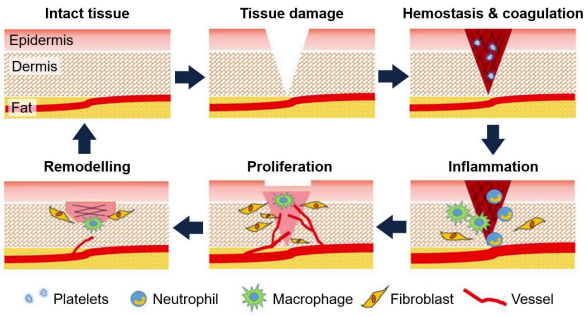
When injury is repaired, or the noxious agent eliminated, inflammation resolves



When injury is repaired, or the noxious agent eliminated, inflammation resolves

Passive mechanisms	Active mechanisms
■ No stimulus - all bacteria are dead, or the wound has been repaired	■ Stop signals produced by the organism
■ Half-life of inflammatory cells	■ Lipid mediators
■ Half-life of inflammatory cytokines	■ Anti-inflammatory cytokine
	■ M2 macrophages

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Inflammation: onset to resolution

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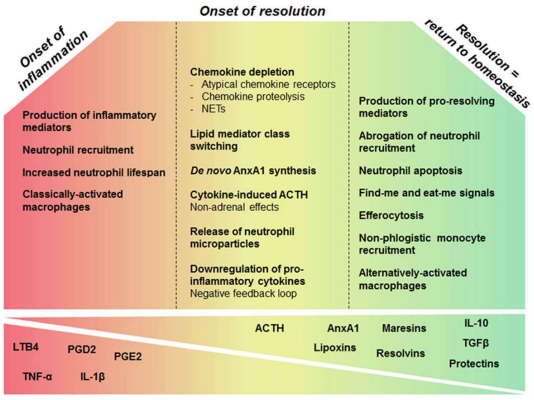

Resolution of inflammation: switch of arachidonate metabolism towards lipoxins

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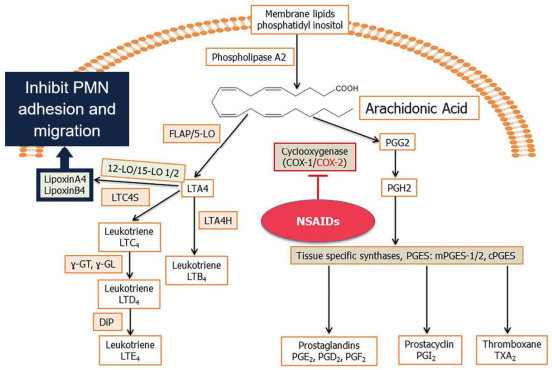

Resolvins

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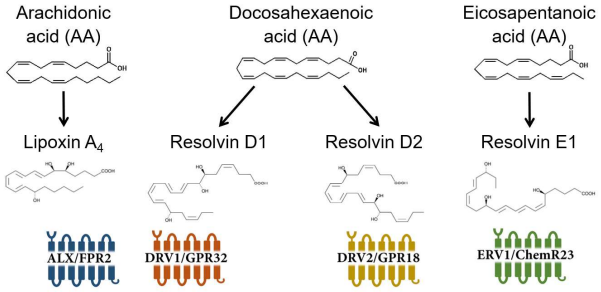

- Inhibit chemotaxis, NF-kB activation and inflammatory cytokines
- Increase non-inflammatory phagocytosis of dead cells
- Induce macrophage polarization towards M2

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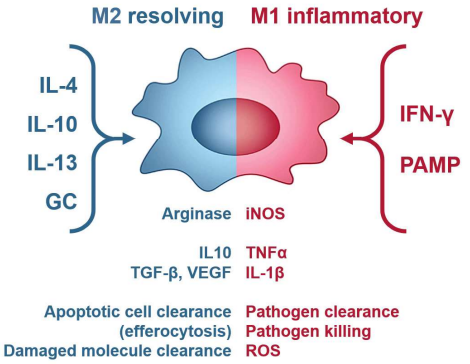
Resolution of inflammation: switch of macrophages from M1 to M2 phenotype



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The role of adaptive immunity in inflammation

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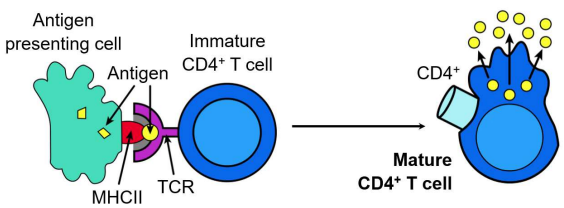
T helper cells regulate inflammation

- Inflammation is normally a **rapid-onset**, early response to infections that precedes the activation of adaptive/specific immunity
- However, adaptive immunity can regulate the inflammatory response through subsets of antigen-specific **T helper (Th) CD4⁺ lymphocytes**



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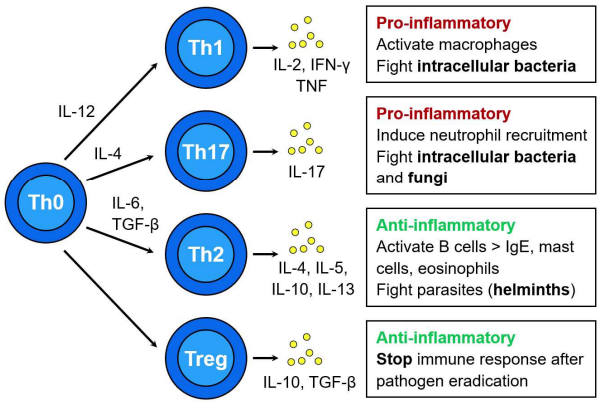
T helper cell phenotypes



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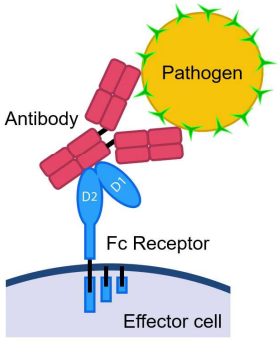
B cells can regulate inflammation



- Antibodies produced as a result of **antigen-specific B-cell activation** can regulate inflammation
- The antibody **Fc region** binds to **Fc receptors**
- This is important for phagocytosis but can also regulate inflammatory cells

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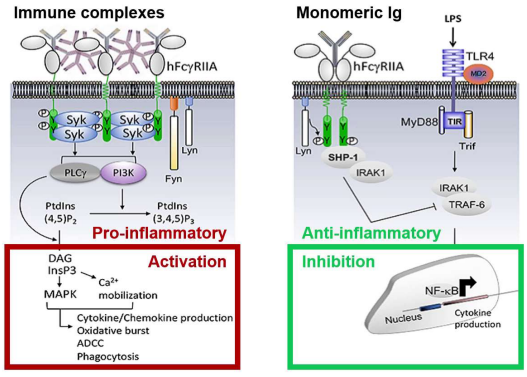
IgG binding to FcγR regulates the production of inflammatory cytokines and ROS



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FcγRs are pro- and anti-inflammatory

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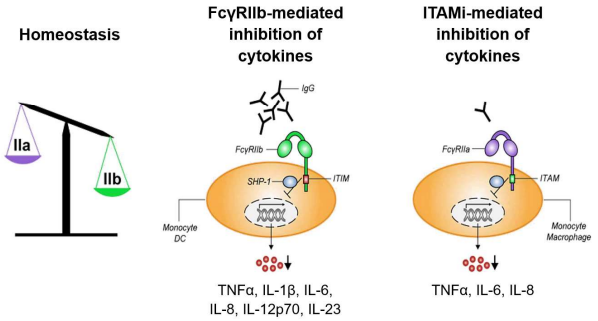
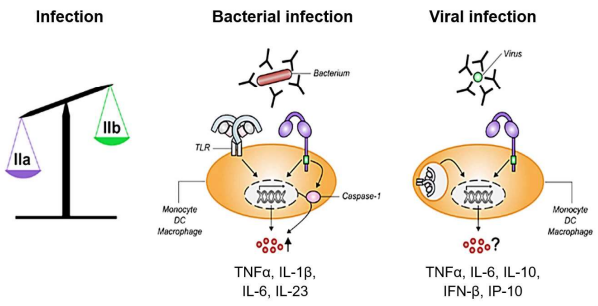
FcγRIIb: anti-inflammatory | **FcγRIIa: pro-inflammatory**



FcγRs are pro- and anti-inflammatory

FcγRIIb: anti-inflammatory | **FcγRIIa: pro-inflammatory**



IgG glycosylation patterns

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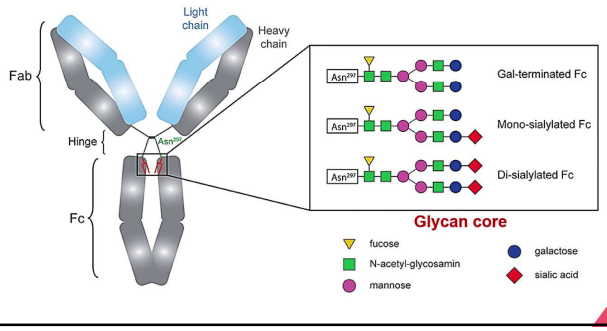
The glycosylation pattern of IgGs can also determine their pro- or anti-inflammatory activity (e.g. sialylation -> anti-inflammatory IgGs)



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Resolution of inflammation: chronic inflammation

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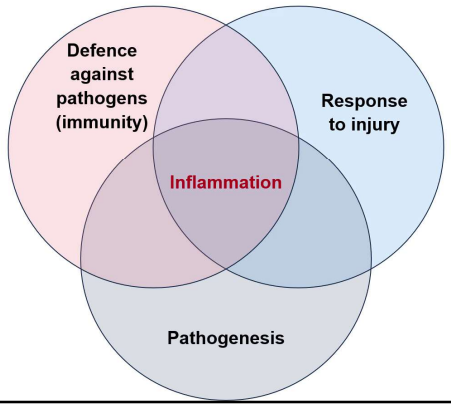
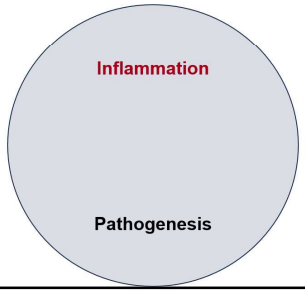
The inflammatory response can be discussed in different-contexts



The inflammatory response can be discussed in different-contexts



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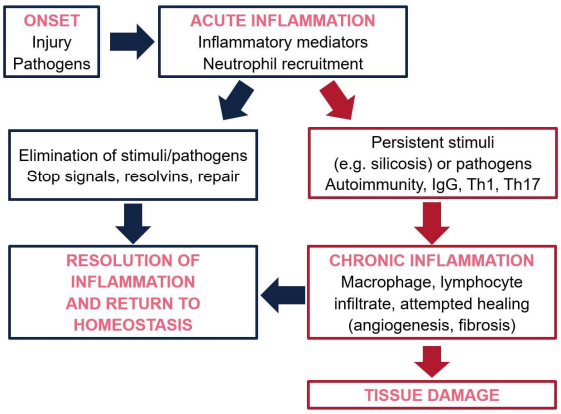
Inflammation in pathogenesis



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Chronic inflammation is not just about duration

Acute inflammation

Inflammatory mediators, neutrophil recruitment

Chronic inflammation

Macrophage, lymphocyte infiltrate, attempted healing (angiogenesis, fibrosis)

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Differences between acute and chronic inflammation

	Acute	Chronic
Time of onset	Minutes/hours	Days
Duration	Short-term (days, weeks)	Persistent (months, years)
Cell infiltrate	Neutrophils	Mono/mac, lymphocytes, plasma cells
Magnitude	+++	+
Results	Removal of trigger, repair	Collateral damage
Biomarkers	IL-6, CRP	low CRP (need hsCRP)
Key cytokines	IL-1, IL-6, TNF, chemokines	IL-12, IL-17, IFN-γ
Systemic effects (acute-phase)	+++	+
Prevalent site	Vessels	Tissues
Fibrosis, angiogenesis	+	+++
Tissue damage	+	+++

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Hallmarks of inflammation

- Calor (heat)
- Rubor (redness)
- Tumor (swelling)
- Dolor (pain)

Functio lesa (impairment of function)

Tissue damage can be due to the same mediators involved in host defence (TNF, reactive oxygen species, proteases)

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Types of mechanism that let us survive an infection

Pathogen control

Decreases the number of pathogens (killing or growth inhibition)

Damage control

Protects from the damage induced by the infection

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Types of mechanism that let us survive an infection

Pathogen control

Decreases the number of pathogens (killing or growth inhibition)

Damage control

Protects from the damage induced by the infection

Protects from the damage induced by the "friendly fire" of immunity

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Types of mechanism that let us survive an infection

- This is arguably the most successfully developed field of **immunopharmacology**
- Nowadays, the top sale in biologics are antibodies that inhibit specific inflammatory cytokines (e.g. IL-1, IL-6, TNF), that are used for the therapy of chronic inflammatory diseases

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Thanks for your attention!

The author does not have any financial interest to disclose

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