Inflammation

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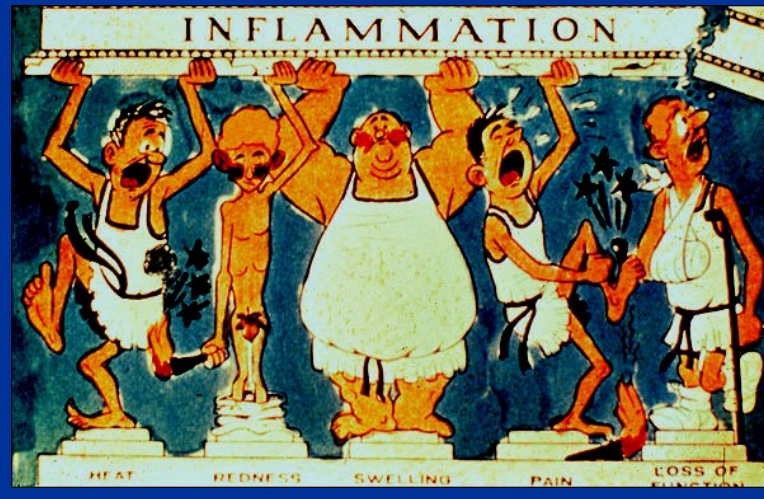


A process known from ancient times, characterized by “cardinal signs”.

But WHY and HOW this process is activated?

2

INFLAMMATION

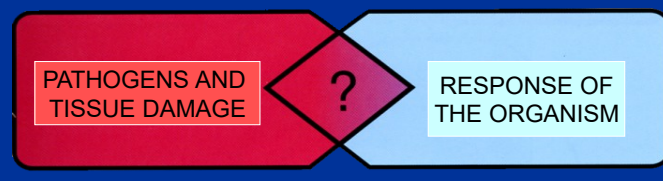
INFLAMMATION IS A DEFENSE MECHANISM THAT CAN BE DEFINED AS "INNATE" (NOT ADAPTIVE)

– It is activated by cellular and tissue damages or signals caused by biological, chemical or physical insults

–The process is directed towards:

1) the elimination of the initial cause of the damage

2) the activation of tissue regeneration and healing



3

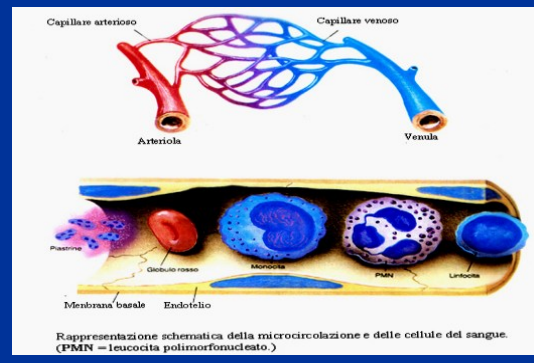
Causes of inflammation

- Microorganisms (bacteria, fungi, virus, worms, protozoa, insects)
- Toxic Agents
- Mechanical Agents (trauma)
- Thermical Agents
- Radioactivity
- Endogenous Toxins (uremia)
- Tumors
- Necrosis

4

Inflammation as a Process

Inflammations is characterized by a dynamic sequence of phenomena that lead to a significant vascular reaction (at the level of the microcirculation) characterized by movement of fluids and leucocytes from the blood to the extravasal tissue.



Rappresentazione schematica della microcircolazione e delle cellule del sangue. (PMN = leucocita polimorfonucleato.)

These events favor the action of components of the immunitary system on the site of the damage and activate the tissue reconstruction and healing of the damaged regions.

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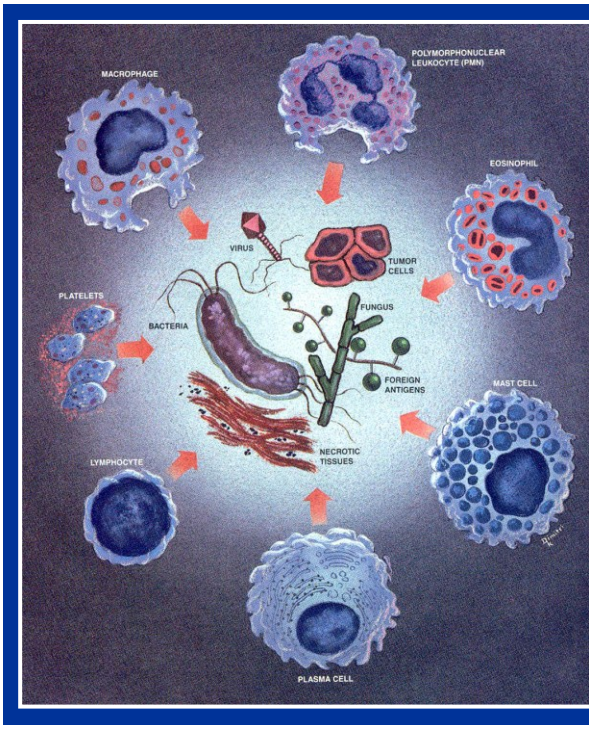
Inflammation

- Inflammation is a protective response of connective tissue to injury.
- Inflammation starts with recognition (sensing) of the injury and activation of endothelial cells and white blood cells.
  - Chemical mediators
  - Changes in vessels
  - Cellular events

6



Cells involved in the inflammatory processes

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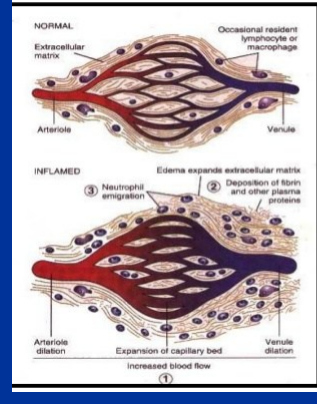
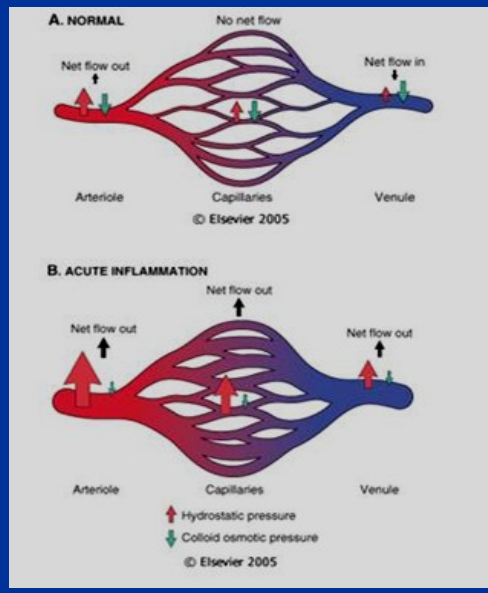
MechanismInflammation



1. Vaso dilatation
2. Exudation - Edema
3. Emigration of cells
4. Chemotaxis

8

Vascular Changes



- increased vascular permeability and vessel dilatation

Colloid or oncotic pressure is the "pulling pressure" into a vessel. Depends on the protein concentration in the liquids. A higher protein content in the blood pulls more fluid into the vessel

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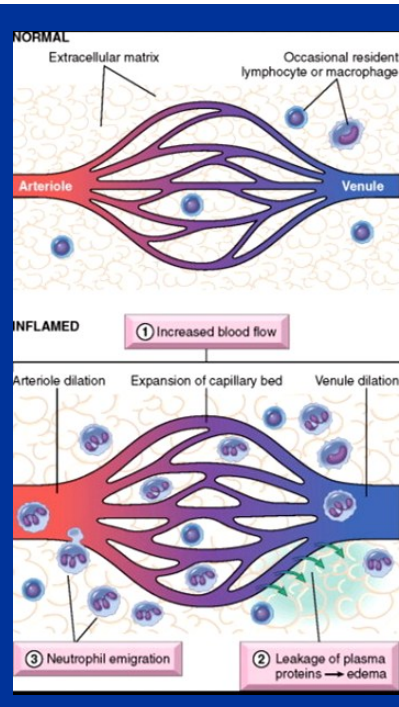

- increased vascular permeability and vessel dilatation,
- a protein rich fluid (exudate) from the blood entering the tissue,
- cellular infiltrate: the early arrival of neutrophils replaced later by macrophage and occasional lymphocytes, then fibroblasts for healing

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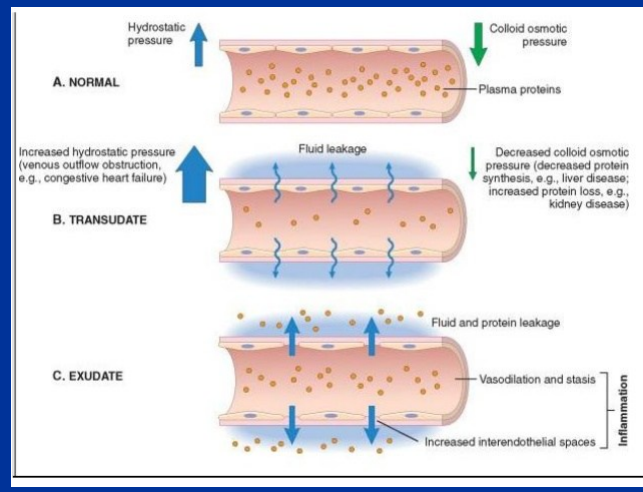
Hydrostatic pressure
Colloid osmotic pressure
A. NORMAL
Plasma proteins
Increased hydrostatic pressure (venous outflow obstruction, e.g., congestive heart failure)
Fluid leakage
Decreased colloid osmotic pressure (decreased protein synthesis, e.g., liver disease; increased protein loss, e.g., kidney disease)
B. TRANSUDATE
Fluid and protein leakage
C. EXUDATE
Vasodilation and stasis
Inflammation
Increased interendothelial spaces

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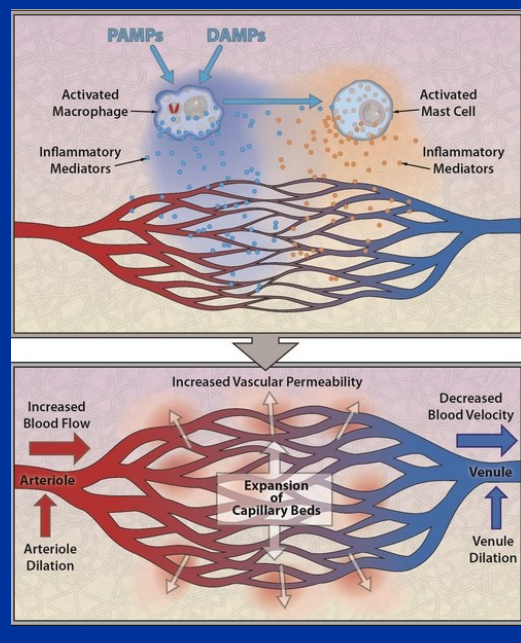
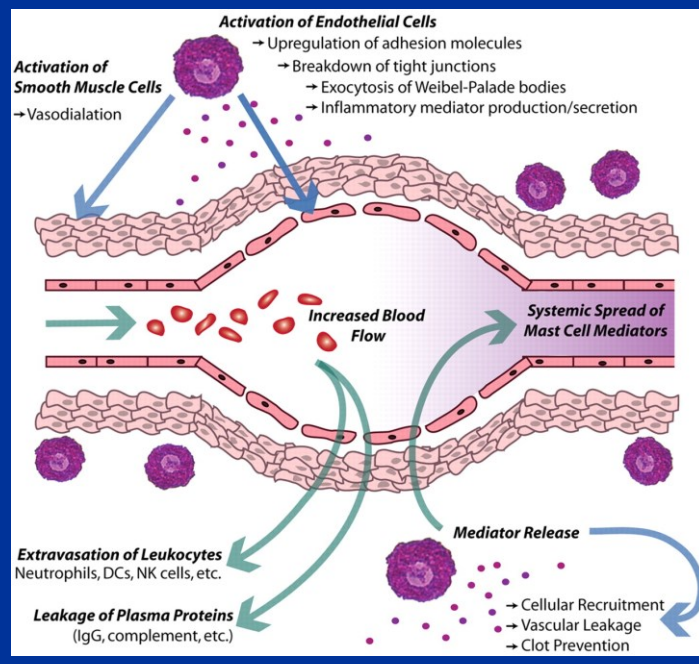

PAMPs
DAMPs
Activated Macrophage
Activated Mast Cell
Inflammatory Mediators
Inflammatory Mediators
Increased Vascular Permeability
Increased Blood Flow
Decreased Blood Velocity
Arteriole
Venule
Expansion of Capillary Beds
Arteriole Dilation
Venule Dilation

12



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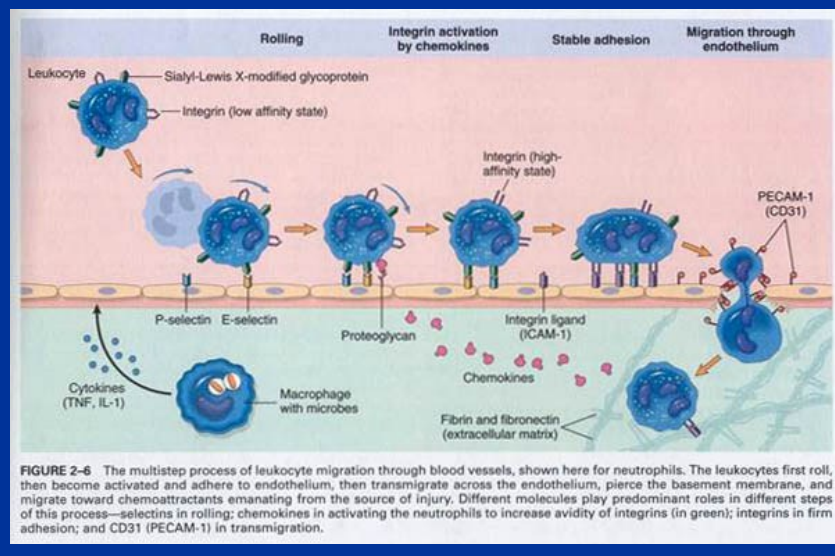

FIGURE 2–6 The multistep process of leukocyte migration through blood vessels, shown here for neutrophils. The leukocytes first roll, then become activated and adhere to endothelium, then transmigrate across the endothelium, pierce the basement membrane, and migrate toward chemoattractants emanating from the source of injury. Different molecules play predominant roles in different steps of this process—selectins in rolling; chemokines in activating the neutrophils to increase avidity of integrins (in green); integrins in firm adhesion; and CD31 (PECAM-1) in transmigration.

cellular infiltrate: the early arrival of neutrophils replaced later by macrophage and occasional lymphocytes, then fibroblasts for healing

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Initial Results of Tissue Injury

- damaged tissue activates plasma enzyme systems
- broken or damaged blood vessels clot
- activated cells release proinflammatory mediators
  - preformed: histamine, serotonin, lysosomal enzymes
  - newly synthesized: cytokines, lipid products, nitric oxide
- neurological responses (pain)
  - pressure, damaged nerves, prostaglandins, bradykinin

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The Five Cardinal Signs of Acute Inflammation

CALOR RUBOR TUMOR DOLOR FUNCTIO LESA

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Other clinical signs associated with inflammation

- mucus production
  - (mast cells-histamine-mucus glands)
- smooth muscle contraction (spasmogens), e.g. bronchoconstriction
- systemic acute phase reactions
  - elevated ESR (erythrocyte sedimentation rate)
  - iron is sequestered ("anemia of inflammation")
  - fever (cytokines)

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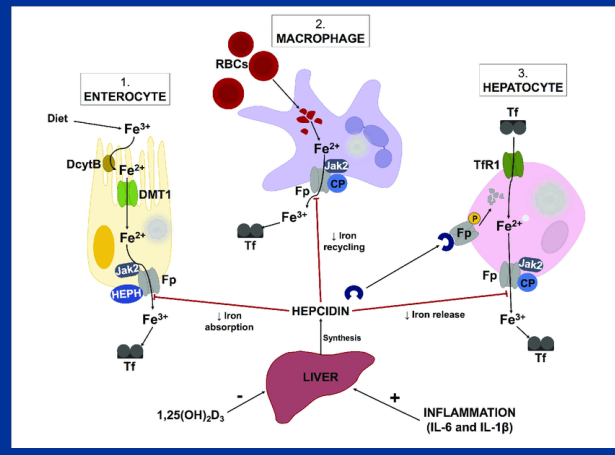

Infection and inflammation are associated with increased hepcidin production and acquired anemia of inflammation.
The upregulation of hepcidin by inflammatory stress response pathways is a major critical event triggering systemic iron withdrawal and sequestration due to its down-regulation of ferroportin. Reduced ferroportin levels limit dietary iron absorption and promote iron retention by the RES
Hepcidin binds to ferroportin inducing its internalization and degradation

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How iron is sequestered ("anemia of inflammation")

Iron levels must be tightly regulated to provide an essential nutrient that is involved in oxygen delivery, metabolism and redox regulation while guarding against excessive levels of a primary toxicant that can generate reactive oxygen species (ROS) to produce cellular damage and death.

Most of the iron necessary to fulfill primary needs of the body is recycled from senescent red blood cells by the reticuloendothelial system (RES; i.e. duodenal enterocytes and macrophages). The conservation of iron is offset by additional nutritional and/or environmental demands (e.g., pregnancy, blood loss, hypoxia, etc.) to ultimately dictate the body's total iron burden. This fine-tuning is primarily adjusted through iron absorption by duodenal enterocytes.
Iron deficiency evolves during the anemia of inflammation or chronic disease by limiting iron absorption and retaining the metal in the RES.

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ACCORDING TO THE PERSISTANCE OF THE PROCESS, INFLAMMATION CAN BE DEFINED AS:

ACUTE: THE PROCESS IS EXTINGUISED IN HOURS OR IN FEW DAYS

CHRONIC: THE PROCESS PERSISTS FOR WEEKS OR LONGER

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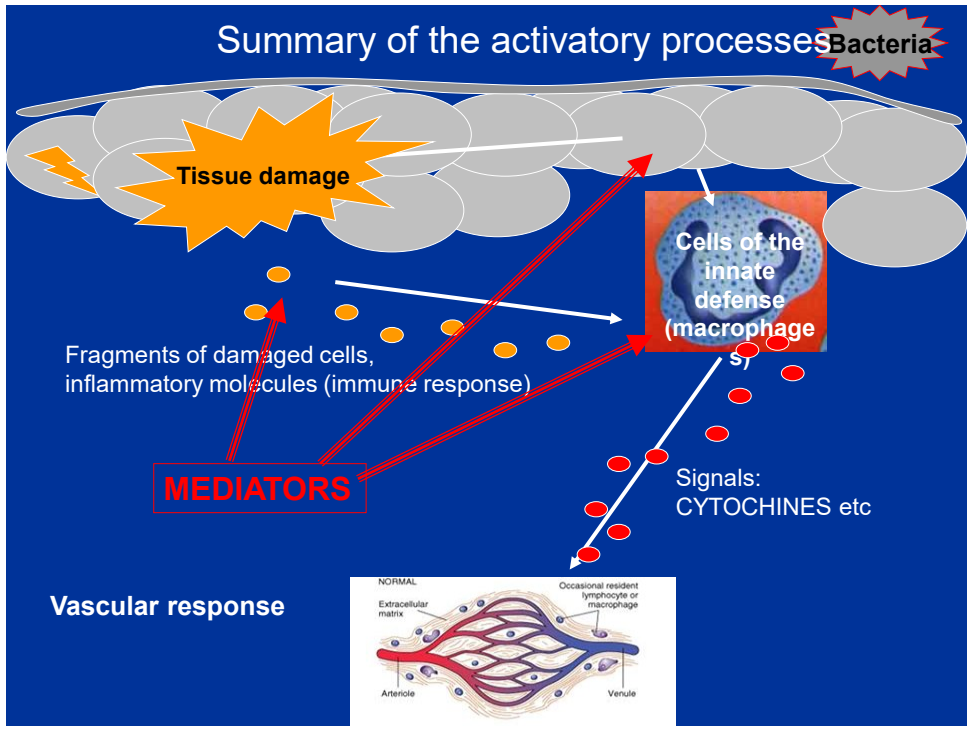

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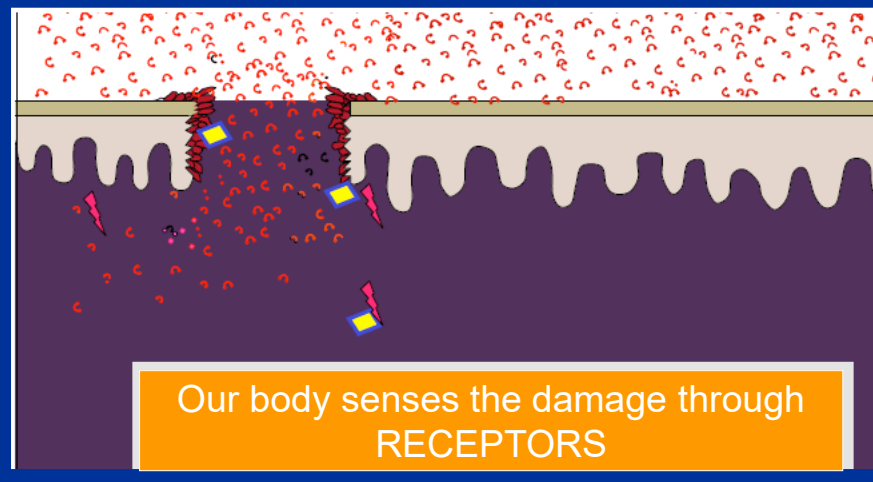
Receptors of the immune response and of inflammation

The immune system and the innate immunitary responses share with the inflammatory processes not only cells and mechanisms, but also RECEPTORS

22



Our body senses the damage through RECEPTORS

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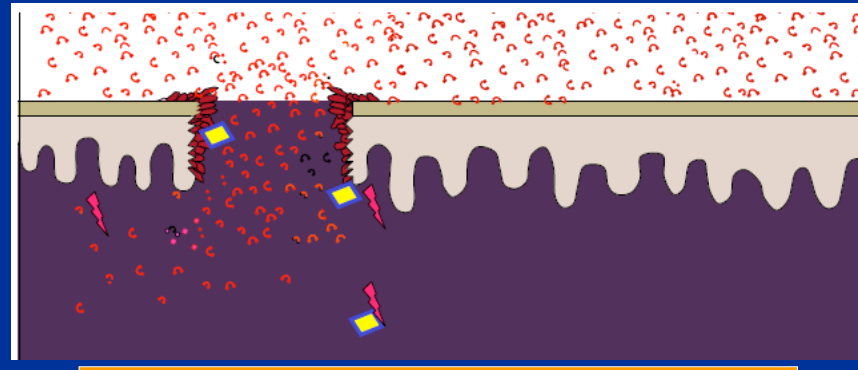
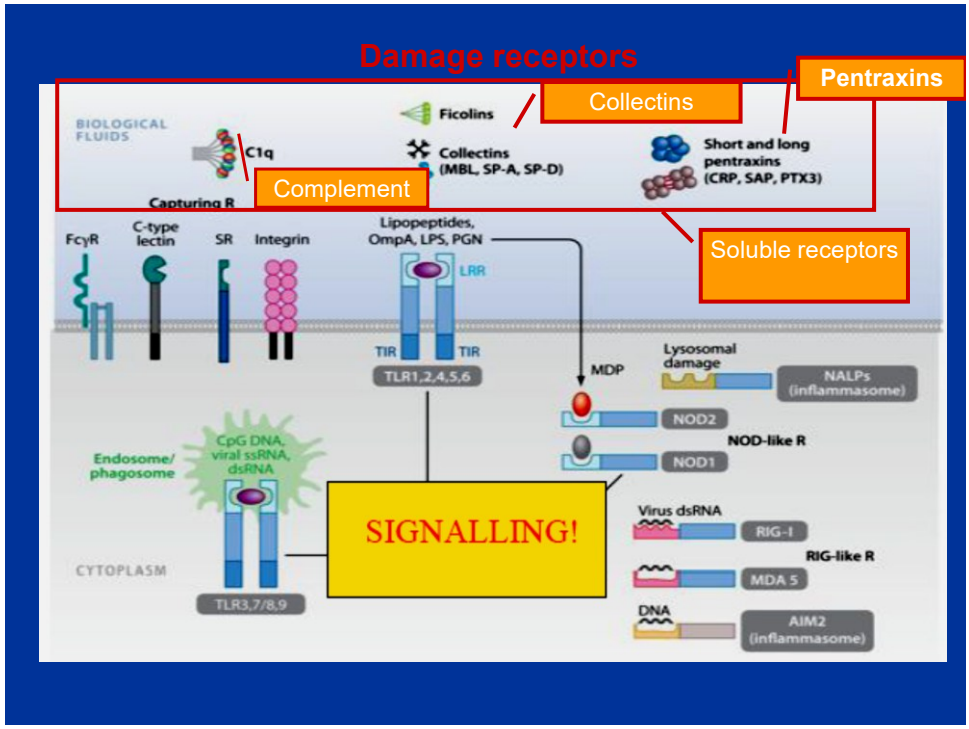


We have receptors that can be distinguished in:

- Soluble Receptors
- Membrane receptors
- Cytoplasmic receptors

24



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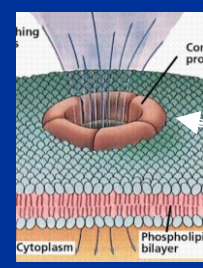
The complement system

The complement system is an essential element of the defense mechanisms against infectious agents.
It is constituted by a group of circulating and membrane proteins, capable of interacting with each other and with cell membranes.
The activation cascade of its soluble proteins (ie C1, C2 ... C9) is the basis of meccanism of cell lysis: these are introduced in the membranes of pathogens causing pores on them leading to lysis.
During activation of the complement there is also the recruitment and activation of various immune system cells (lymphocytes).
There are three different mechanisms of activation:

a) Classic pathway

b) Alternative pathway

c) Lectins pathway



PORUS,
Complements proteins
C5b, C6, C7, C8, C9

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Complement's two major functions:

1. Alter biological membranes to cause direct cell lysis or enhanced susceptibility to phagocytosis.

2. Promote the inflammatory response.

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General Properties of Complement

- Primary role is cell lysis.
- Activity of complement destroyed by heating sera to 56 C for 30 minutes.
- IgM and IgG are the only immunoglobulin capable of activating complement (classical pathway).
- Complement activation can be initiated by complex polysaccharides or enzymes (alternative pathway).
- Portions of the complement system contribute to chemotaxis, opsonization, immune adherence, anaphylatoxin formation, virus neutralization, and other physiologic functions

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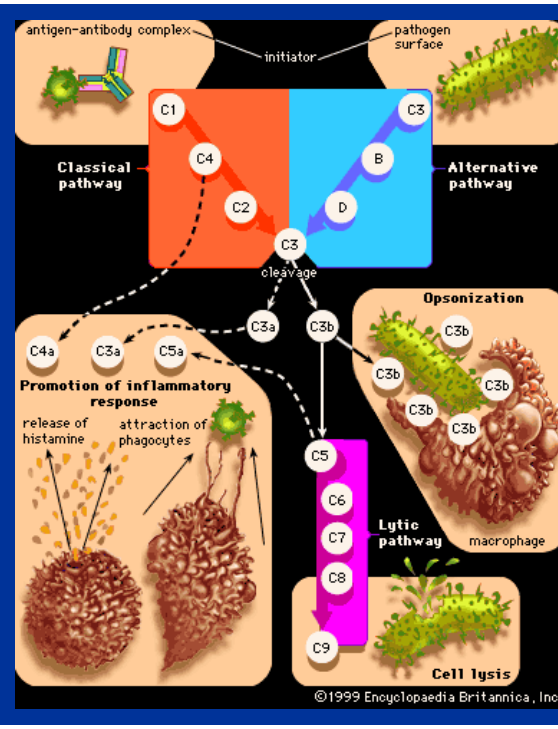
C1q is part of the C1-complex. C1q can bind to the antibodies IgM and IgG

Complement



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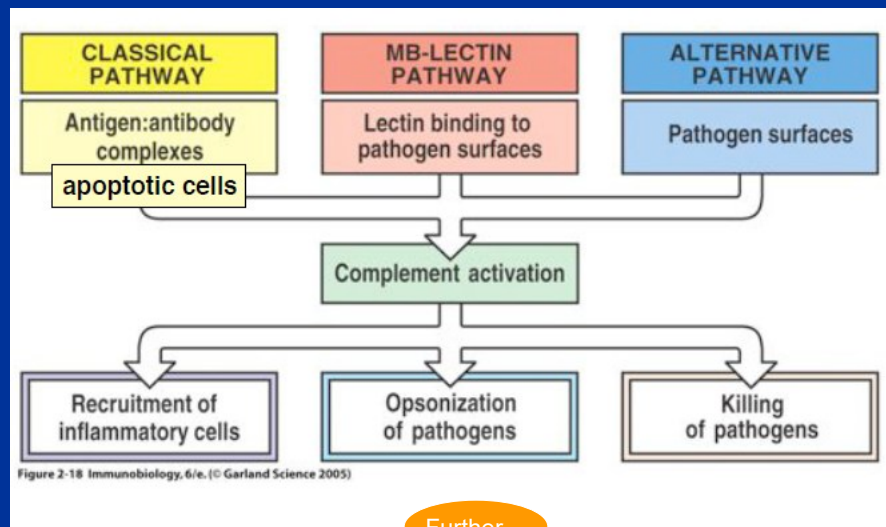
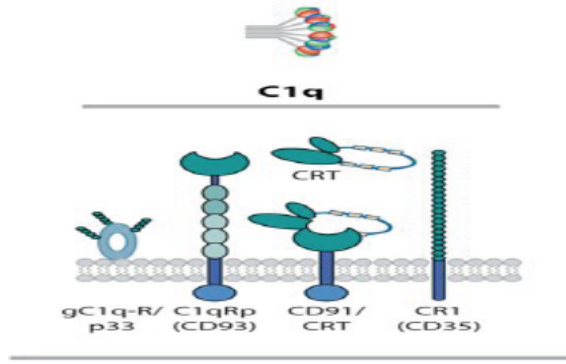


Further…

30

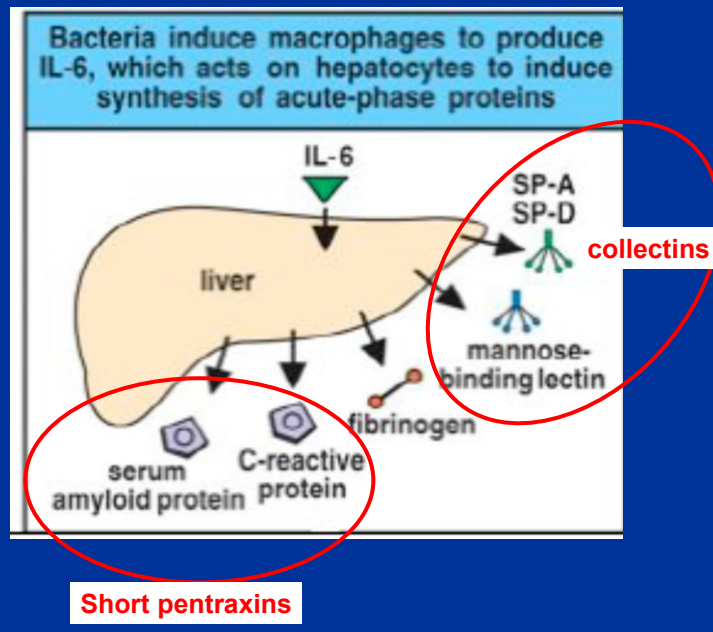
C1q binds to a wide range of cell types (PMN, monocytes, lymphocytes, DCs, ECs, and platelets), resulting in the induction of cell-specific biological responses, which include phagocytosis, chemotaxis, the generation of procoagulant activity, activation of ECs, and enhancement of FcγR- and CR1-mediated phagocytosis and superoxide production.

Receptors for C1q humoral complement factor !



To date, investigators have described four types of C1q-binding proteins/receptors expressed on the cell surface. These include cC1q-R/calreticulin (CRT), a 60-kDa protein ; gC1q-R/p33, a 33-kDa homotrimeric protein; C1q-Rp (CD93), a 120-kDa O-sialoglycoprotein; and CR1 (CD35), the receptor for C3b. In addition to C1q, CRT reportedly serves as a receptor for collectins, such as the MBL, SP-A, SP-D, CL-43, and conglutinin, and, in association with CD91, initiates macropinocytosis and phagocytosis of apoptotic cells

31



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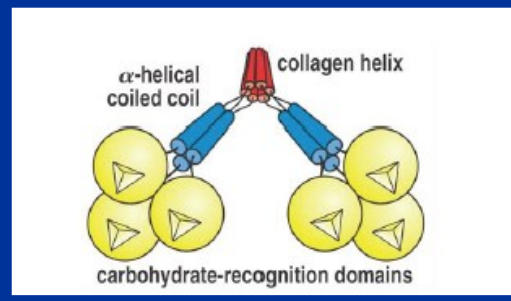
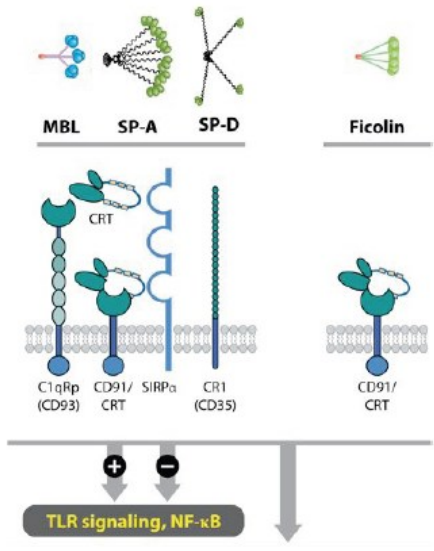
Family of Collectins



MBL
SPA
SPD
FICOLIN

33

Receptors for humoral collectins



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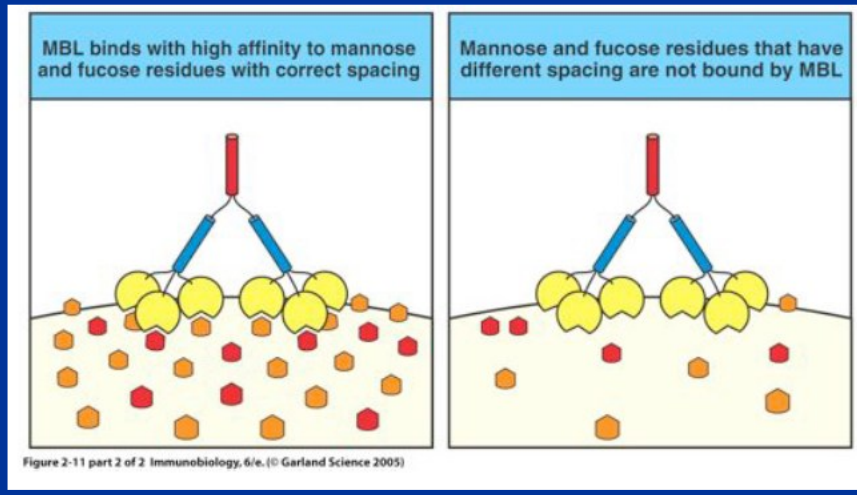
MBL binds mannose and fucose and activate complement and fagocitosis



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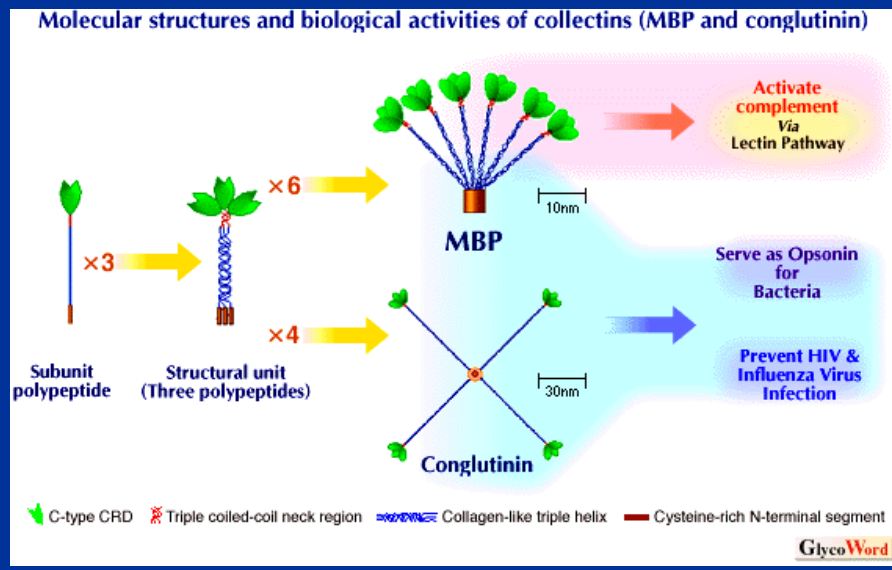

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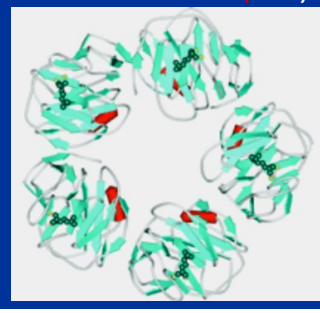
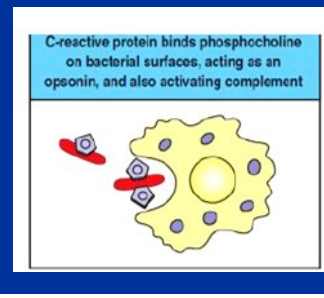
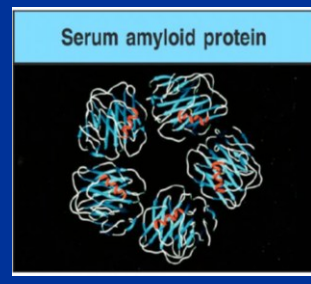
C-Reactive Protein (CRP)





Crystal structure of C-reactive protein complexed with phosphocholine



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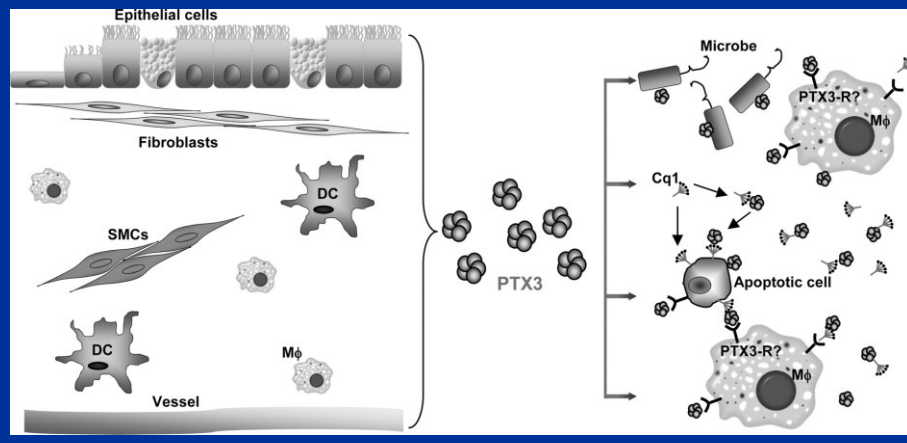
Long pentraxins are produced ouside the liver



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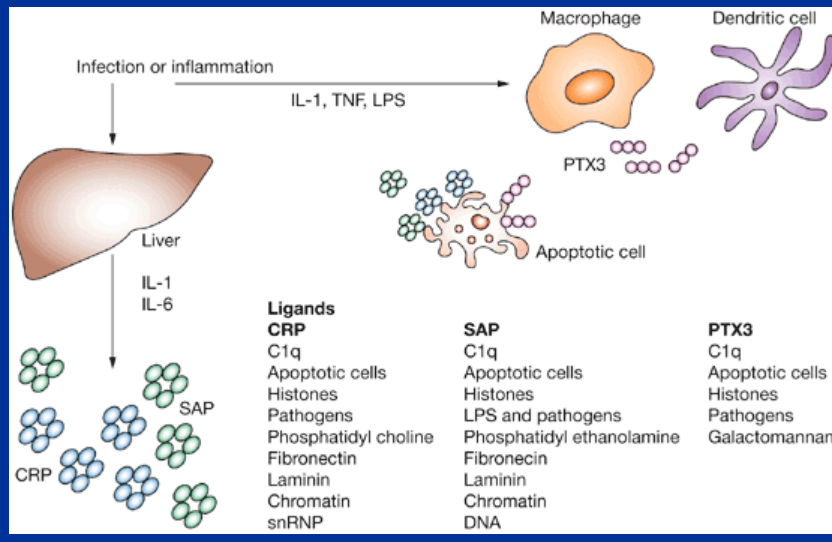
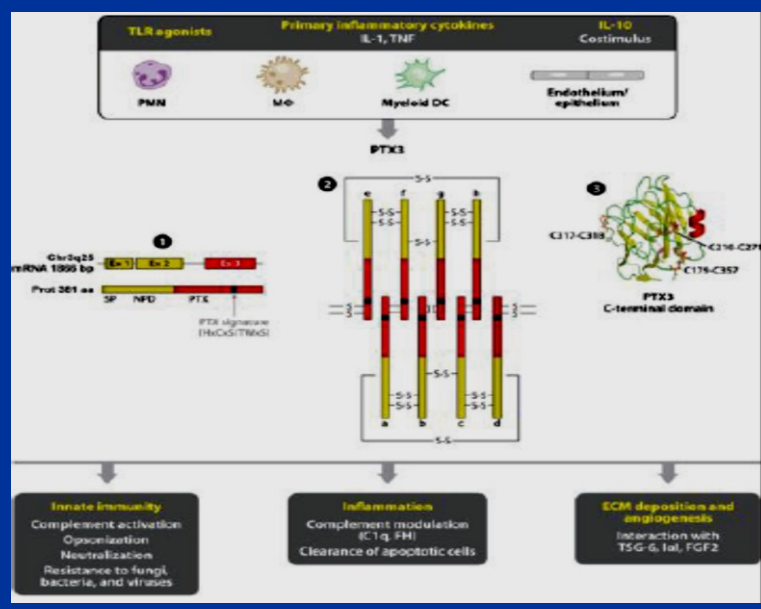
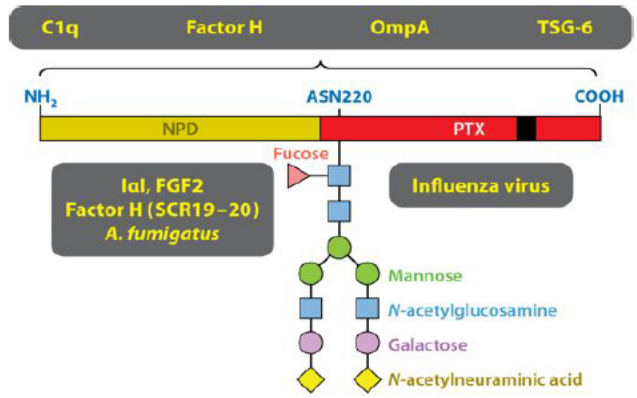

Macrophage
Dendritic cell
Infection or inflammation
IL-1, TNF, LPS
PTX3
Liver
Apoptotic cell
IL-1
IL-6
SAP
CRP
Ligands
CRP
C1q
Apoptotic cells
Histones
Pathogens
Phosphatidyl choline
Fibronectin
Laminin
Chromatin
snRNP
SAP
C1q
Apoptotic cells
Histones
LPS and pathogens
Phosphatidyl ethanolamine
Fibronecin
Laminin
Chromatin
DNA
PTX3
C1q
Apoptotic cells
Histones
Pathogens
Galactomannan

39


TLR agonists
Primary inflammatory cytokines
IL-1, TNF
IL-10
Costimulus
PMN
Myeloid DC
Endothelium/
epithelium
PTX3
Innate immunity
Complement activation
Opsonization
Neutralization
Resistance to fungi,
bacteria, and viruses
Inflammation
Complement modulation
(C1q, FH)
Clearance of apoptotic cells
ECM deposition and
angiogenesis
Interaction with
TSG-6, IaI, FGF2

40

Role of the two PTX3 domains in recognizing ligands.



The overall molecule is necessary for optimal interaction with complement components (C1q and Factor H), microbial moieties (OmpA), and the extracellular matrix protein TSG-6. Recombinant NPD can reconstitute the full-length protein in the interaction with IαI and FGF2. Interaction with conidia of A. fumigatus is also mediated by NPD, whereas influenza virus interacts with the sialic acid present on the glycosidic moiety at Asn220, localized in the PTX domain. (Abbreviations: TSG-6, TNF-α-induced protein 6; NPD, nonpentraxin domain; IαI, inter-α-trypsin inhibitor; FGF2, fibroblast growth factor 2.)

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Several of these receptors are “specific” and recognize conserved microbial structures:

They are defined **Pattern recognition receptors (PRRs)**

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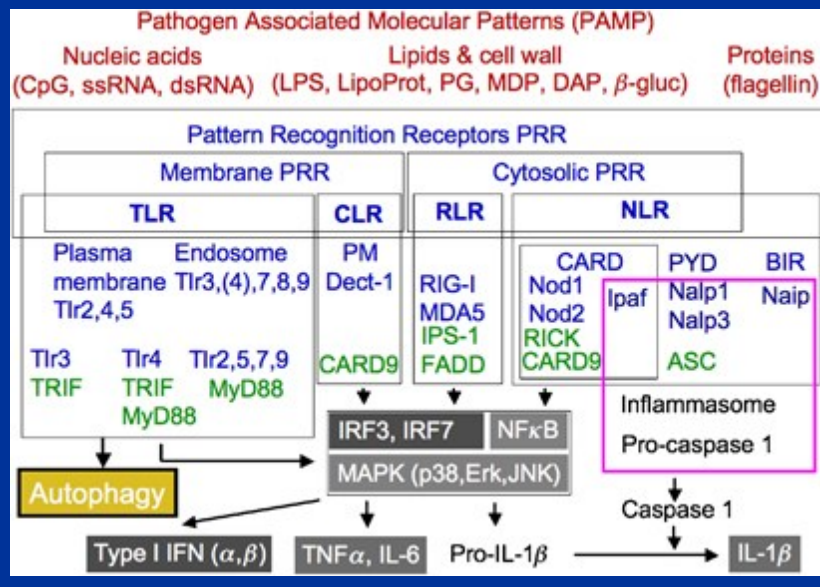

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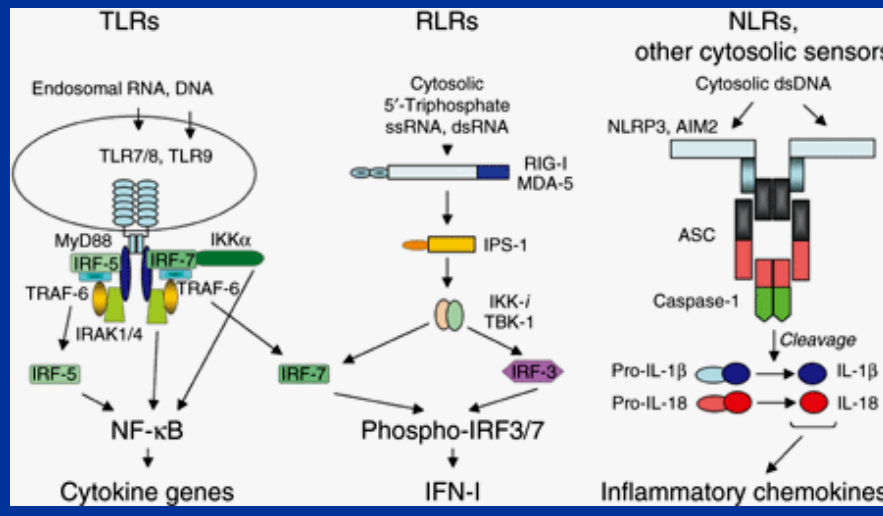
Three classes of pattern recognition receptors implicated in viral nucleic acids recognition in mammalian cells..



NLR, nucleotide oligomerization domain–like receptor; RLR, retinoid acid-inducible gene-I-like receptor; TLR, Toll-like receptor

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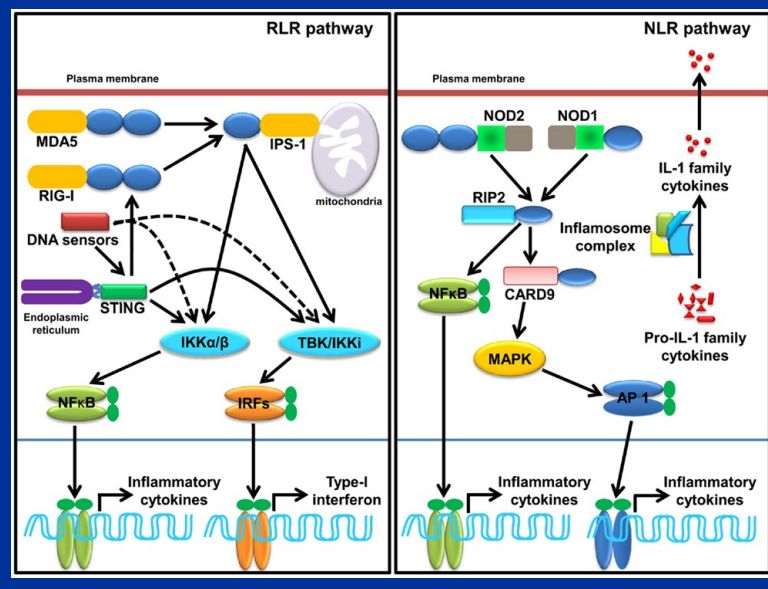

RIG like receptors NOD like receptors

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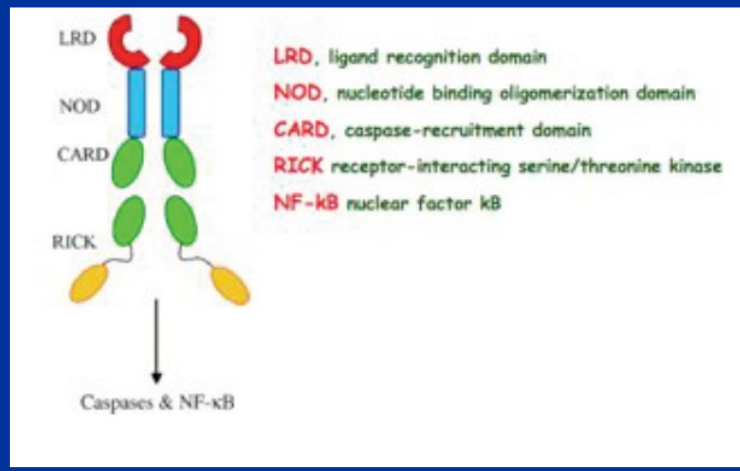

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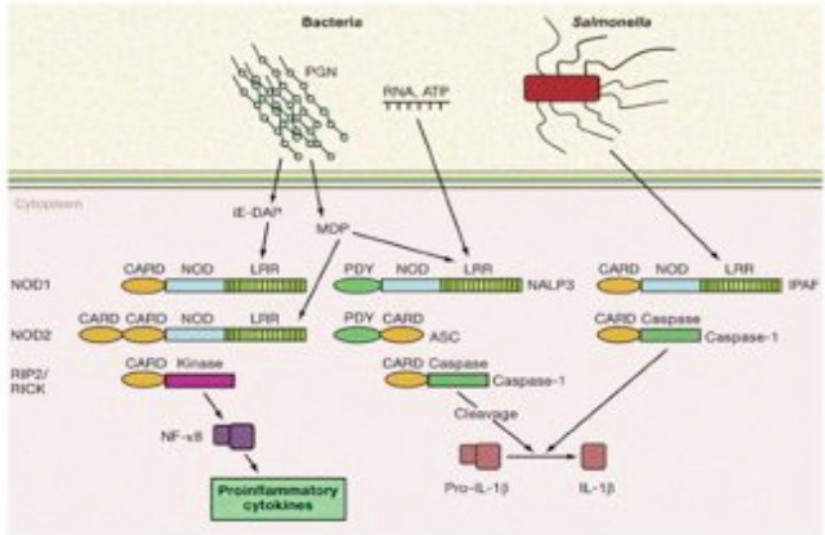
NOD - Pathogens associated molecular patterns recognized in the citosol



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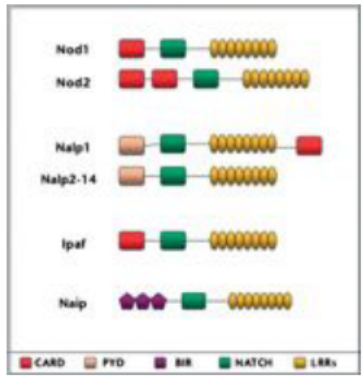
Nalp3 seems to respond to a multitude of agonists from both microbial and host origin, including bacterial RNA and imidazoquinolone compounds, dsRNA from viruses, uric acid, and K + cellular efflux, which can arise from the insertion of a bacterial toxin through the plasma membrane. The fact that Nalp3 seems to be activated in response to uric acid, a host molecule released into the extracellular milieu by necrotic cells, and by potassium efflux, suggest that this NLR may trigger the detection of danger signals, in addition to microbe-derived stimuli.



NLRP3 (also known as NALP3 or cryopyrin) is a known mediator of bacterial and chemical triggers of inflammation, including ATP and uric acid [31]. NLRP3, with its adaptor molecule, apoptotic speck-like protein containing a CARD (ASC) and caspase 1, make up the NLRP3 inflammasome [31]. The NLRP3 inflammasome

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Recognition of RNA viruses by RLRs

The RLR family consists of three members: retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) [1]. RIG-I and MDA5 contain two N-terminal caspase activation and recruitment domains (CARDs), which are essential for their signaling activity. All three molecules have an internal DExD/H-box RNA helicase domain with ATPase activity. This

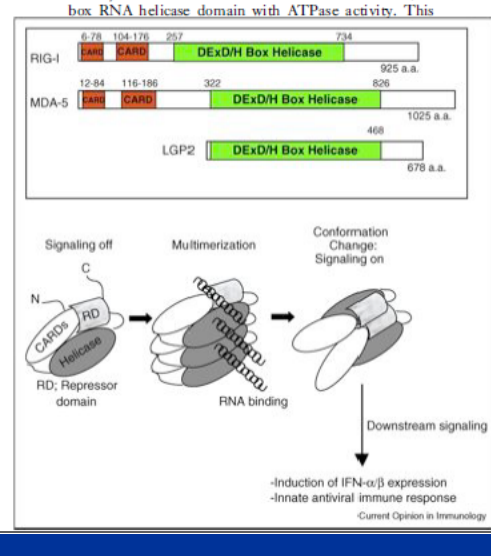

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Table 1

Specificity of RIG-I like receptors for virus recognition

Virus	Genome RNA	Host cytosolic PRR
Vesicular stomatitis virus	Nonsegmented negative-sense, single strand	RIG-I
Respiratory syncytial virus	Nonsegmented negative-sense, single strand	RIG-I
Influenza A virus	Eight RNA segments, negative-sense, single strand	RIG-I
Ebola virus	Nonsegmented negative-sense, single strand	RIG-I
Reovirus	Ten double-stranded segments	RIG-I and MDA5
Hepatitis C virus	Nonsegmented positive-sense, single strand	RIG-I
Dengue virus	Nonsegmented positive-sense, single strand	RIG-I and MDA5
West Nile virus	Nonsegmented positive-sense, single strand	RIG-I and MDA5
Polio virus	Nonsegmented positive-sense, single strand	MDA5

Summarized from Ref. [11].

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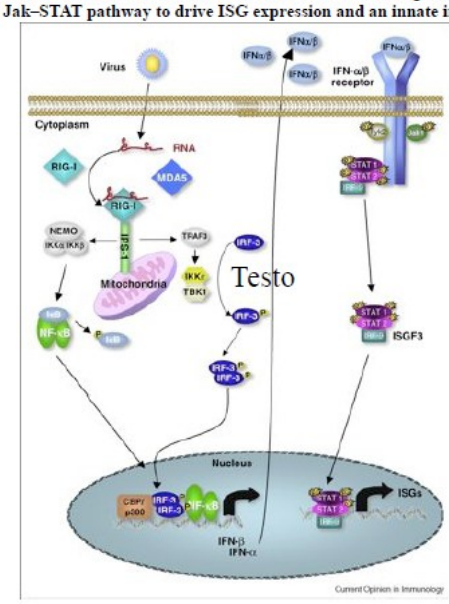
The RLR signaling pathway showing RIG-I bound to ligand RNA and signaling downstream to IRF3 and NF-kB to induce IFN-a/b production from a virus-infected cell. IFN-a/b is then shown signaling through the IFN-a/b receptor and the Jak–STAT pathway to drive ISG expression and an innate immune response.



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Membrane-bound receptors of inflammation and natural immunity

- Receptors for formylated peptides
- C-type lectin receptor (e.g. Mannose)
- Scavenger receptors
- Toll-like receptors
- Immunoglobulin FC receptors
- Complement receptors
- Integrin receptors

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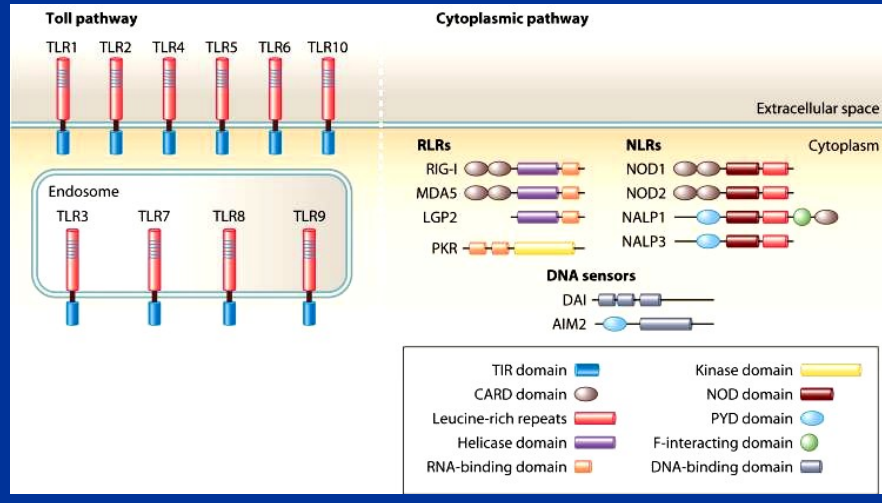

53

Recognition of PAMPs from different pathogens

	Viruses	Gram-positive bacteria	Gram-negative bacteria	Fungi	Protozoa
	GP DNA RNA	DNA LP PG LTA	DNA Porin PG LPS Flagellin	Zymosan Mannan β-glycan	DNA GPI anchors
TLRs:	TLR2, 4 TLR9 TLR3, 7/8	TLR9 TLR2 TLR2 TLR2	TLR9 TLR2 TLR2 TLR4 TLR5	TLR2 TLR2 TLR2,4	TLR9 TLR2,4
RLRs:	RIG-I/MDA5 (PKR)				
NLRs:	NALP3 NALP3	NALP3 NOD2, NALP1/3	NALP3 NOD2, NALP1/3 IPAF		
DNA sensors:	+	+	+		

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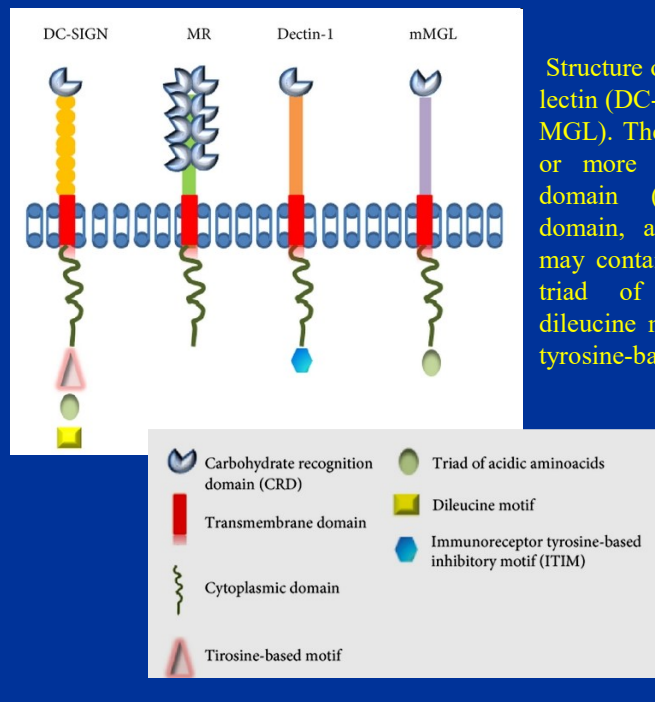

Structure of members of the C-type lectin (DC-SIGN, MR, Dectin1, and MGL). These receptors contain one or more carbohydrate-recognition domain (CRD), transmembrane domain, and cytoplasmic domain may contains tyrosine-based motif, triad of acidic amino acids, dileucine motif or immunoreceptor tyrosine-based inhibitory motif.

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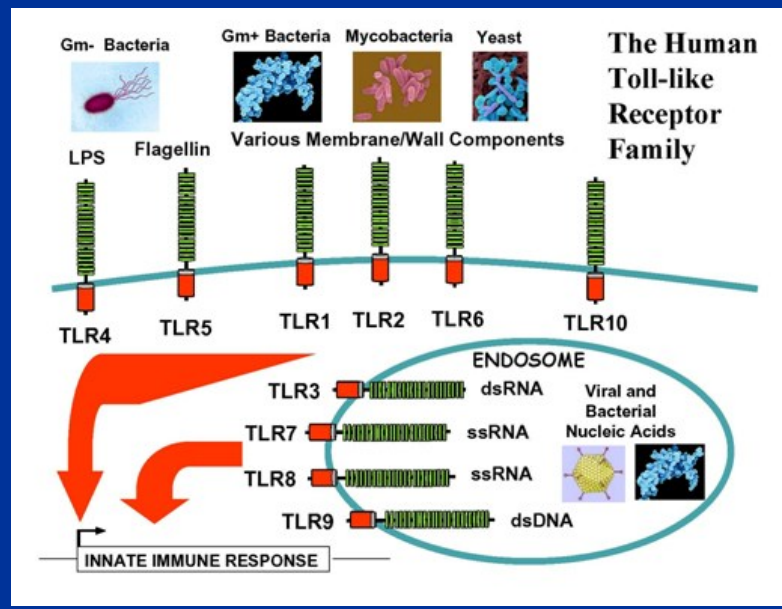

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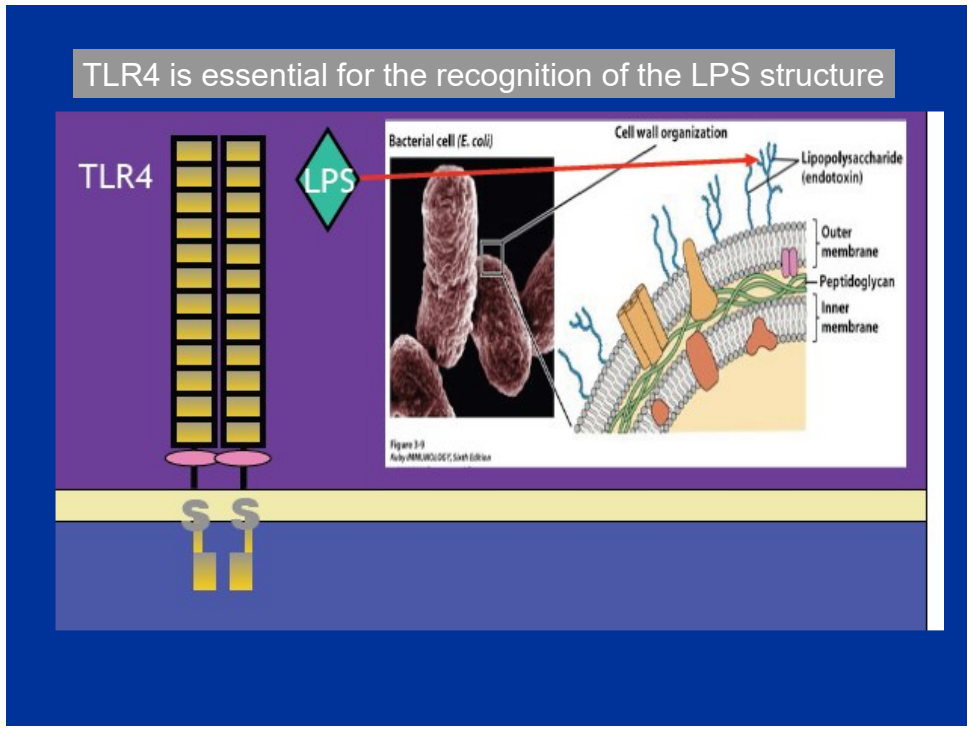
TLR4 is essential for the recognition of the LPS structure
TLR4
LPS
Bacterial cell (E. coli)
Cell wall organization
Lipopolysaccharide (endotoxin)
Outer membrane
Peptidoglycan
Inner membrane

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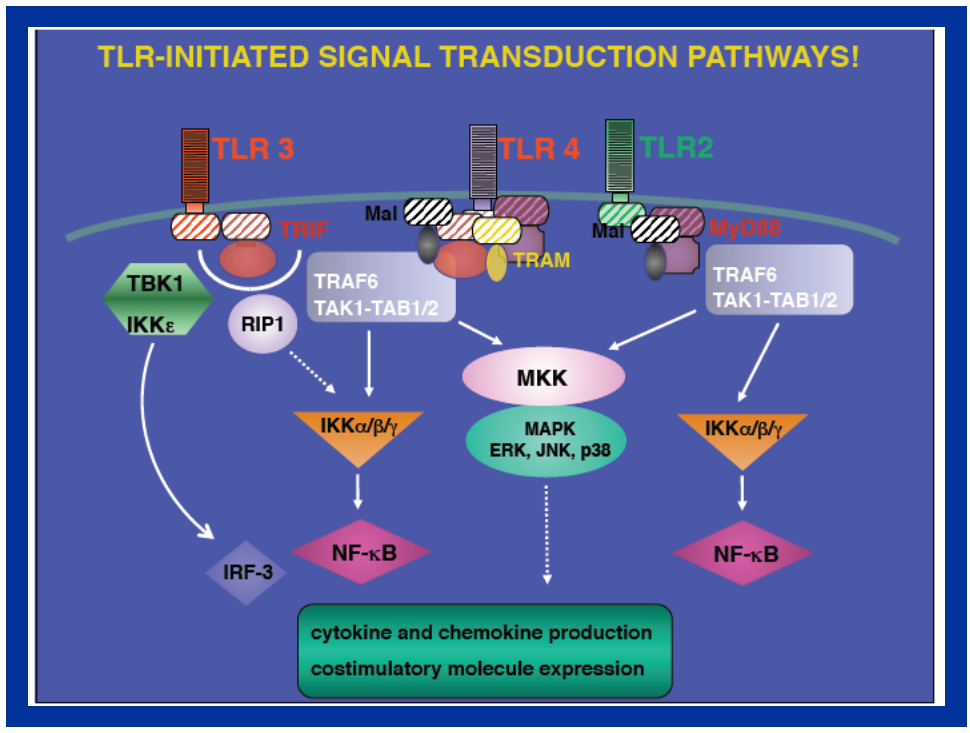
TLR-INITIATED SIGNAL TRANSDUCTION PATHWAYS!
TLR 3
TLR 4
TLR2
TRIF
Mal
Mal
MyD88
TRAM
TBK1
IKKε
RIP1
TRAF6
TAK1-TAB1/2
TRAF6
TAK1-TAB1/2
MKK
MAPK
ERK, JNK, p38
IKKα/β/γ
IKKα/β/γ
IRF-3
NF-κB
NF-κB
cytokine and chemokine production
costimulatory molecule expression

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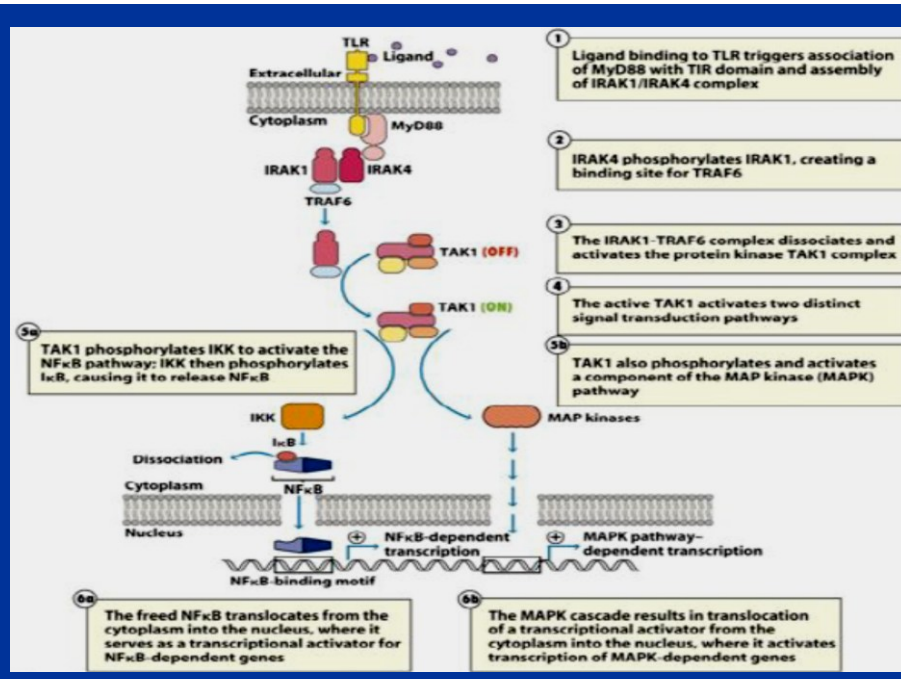

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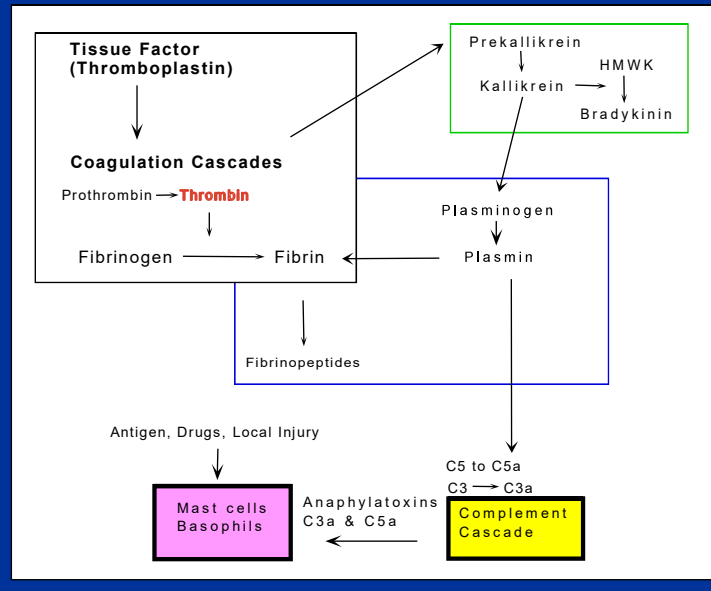
Interrelated Plasma Enzyme Systems Related to Injury

These cascading enzyme systems are interrelated, proinflammatory, and active during tissue injury:

- Coagulation pathways
- Kinin system
- Fibrinolytic reaction
- Complement fixation

60



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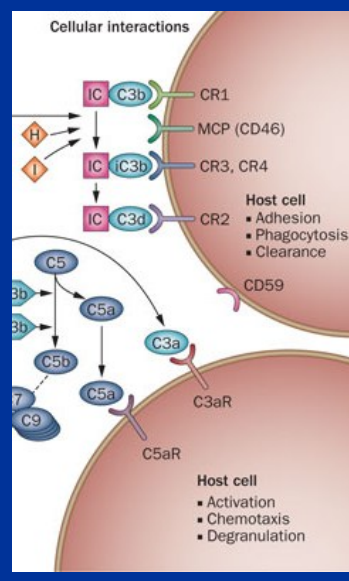
Complement's role in Inflammation



C3a, C4a and C5a are also called **anaphylatoxins:** they cause smooth muscle contraction, histamine release from mast cells, and enhanced vascular permeability.
They also mediate chemotaxis, inflammation, and generation of cytotoxic oxygen radicals.
C4a is less active.

Back..

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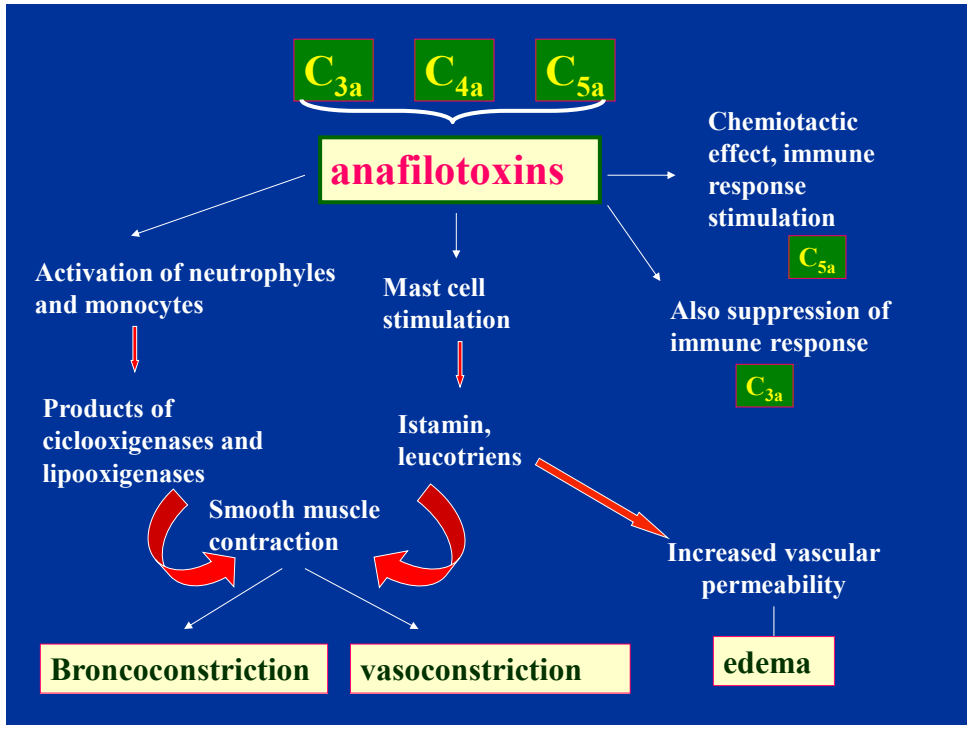

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Biological Activities of Complement

- anaphylatoxins/ vascular permeability agents C3a and C5a; mainly act by histamine release from mast cells
- chemotaxin C5a, also activates lipoxygenases of neutrophils and macrophage, and the release of free radicals thus enhancing the inflammation
- opsonin C3b
- cell lysis by MAC (C5-9)

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Regulation of the Complement Cascade

Modulating mechanisms are necessary to regulate complement activation and control production of biologically active split products

- First means of control is extreme lability of activated complement
  - If activated complement does not combine within milliseconds the activity is lost or decreased.
  - Active fragments are rapidly cleared from the body.

- Second type of control involves specific control proteins
  - C1 inhibitor blocks activity of C1r and C1s.
  - Factor I in activator in the presence of certain cofactors inactivates C3b and C4b.
  - A number of proteins act to control membrane attack unit

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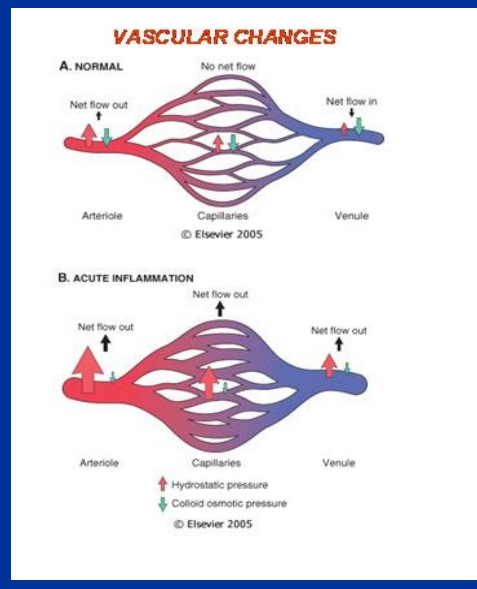
Activation of Endothelium



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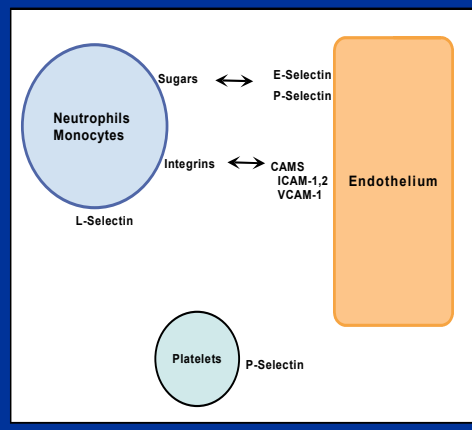
Activation of Endothelium: Receptors



- selectins and addressins (ligands for selectins), and integrins (ICAM and ICAM2) expressed in greater number or active form

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Activated Endothelium Releases Mediators

- PAF (Platelet aggregating factor, thrombosis); IL-1, IL-6/8, TNF-α (liver and brain); reduced amounts of NO (decreases granulocyte and platelet adherence and vascular tone)
- release of alpha chemokines attracts macrophage, e.g. macrophage chemotactic peptide-1; and beta chemokines, e.g. IL-8 are chemotactic for granulocytes.

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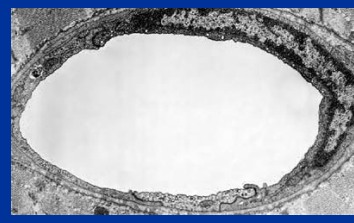
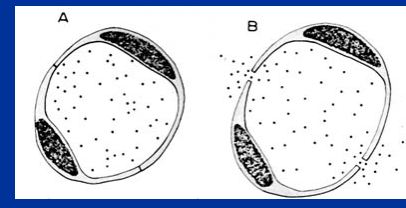
Activation of Endothelium: Morphology





- retraction of activated endothelium widens junctions between cells and allowing fluid loss
- fluid creates edema and effusion
- loss of fluid raises the protein concentration in capillary plasma

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Endothelial Retraction Effects

- vWF (Von Willebrand factor (vWF) is a blood glycoprotein involved in haemostasis) is released, subendothelial collagen is exposed
- platelets and fibrinogen adhere
- Damaged tissue releases Tissue Factor (thromboplastin), to initiate the cascade
- Thrombin is formed
  - converts the soluble fibrinogen into insoluble fibrin
  - direct effects on a variety of cells, e.g. activating endothelium
  - activates Complement.

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Endothelium enters a "Procoagulant State"

- reduced levels of factors to discourage platelet aggregation and adhesion create a "procoagulant state" for the endothelium
  - No longer releases factors PGI2, NO; thrombomodulin decreases, procoagulant tissue factor increases.
- Retraction leaves the subendothelial matrix exposed, to which platelets may pavement
  - onto the collagen/ vWF as does Hageman Factor (Factor XII).
- Aggregated platelets release proinflammatory factors.
  - PAF, ADP; serotonin...

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When Severe Damage of Endothelium Occurs

- Severely damaged vessels can lose endothelium
- thrombi form, may infarct
- healing takes longer

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Platelets

- **adhere** to exposed collagen & VWF, and contribute to the hemostatic plug occluding small vessels.
- **aggregate** and adhere in response to:
  - thrombin, collagen, immune complexes, PAF

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Platelet Released Mediators

- cell growth factors
- serotonin and **histamine** (increased vasodilatation and permeability)
- **epinephrine** (release of neutrophils)
- **Ca^{++}** needed in the coagulation sequence, **ADP & PAF**(a potent agent of vasodilatation and increased permeability and platelet aggregation)
- **TXA2** (facilitates aggregation and vasoconstrictor)

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Blood Clot Terminology

- **Hemostasis** is a response to vascular injury, and leading to arrest of the hemorrhage, involving vasoconstriction, tissue swelling, the coagulation cascade, and thrombosis.
- **Coagulation** is the conversion of soluble plasma fibrinogen to insoluble fibrin polymer as catalyzed by the protease thrombin, and resulting from a cascade of reactions.
- **Thrombosis,** a blood clot in the circulation, is an aggregate of coagulated blood containing platelets, fibrin, erythrocytes, and leukocytes.

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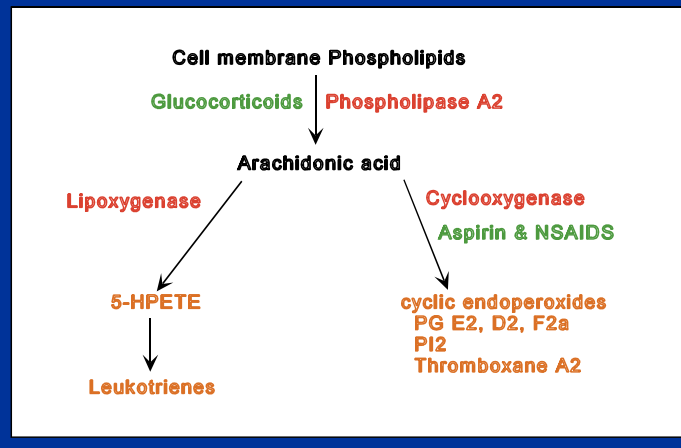
Lipid Mediators of Inflammation



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Arachidonic Acid Metabolites

Phospholipase A_2 releases arachidonic acid from membrane phospholipids (inhibited by steroids), and goes into:

- lipoxygenase pathway
  - 5-HETE (chemotactic)
  - LTB4: chemotactic for granulocytes and macrophage
  - LTC4, LTD4, LTE4: potent vasopermeability and bronchial spasm
- cyclooxygenase pathway (inhibited by aspirin, indomethacin)
  - PGI2: vasodilation, inhibits platelet aggregation
  - TXA2: vasoconstriction, promotes platelet aggregation
  - PGD2, PGE2, PGF2α: vasodilation
- lipoxins (platelet 12-lipoxygenase on neutrophil LTA4)
  - LXA4 vasodilation, reduces LTC4 vasoconstriction

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Inhibition of Arachidonic Acid Metabolism

- glucocorticoids: inhibit genetic expression of cyclooxygenase, cytokines e.g. (IL-1 and TNF-α) , and iNOS, and upregulate anti-inflammatory proteins e.g., lipocortin 1, an inhibitor of PLA2
- dietary fish oil, linoleic acid, makes less potent mediators than those from AA.
- specific COX-2 inhibitors
  - COX: aspirin, indomethacin, etc.
  - COX-1 (homeostatic) and COX-2 (inflammatory)

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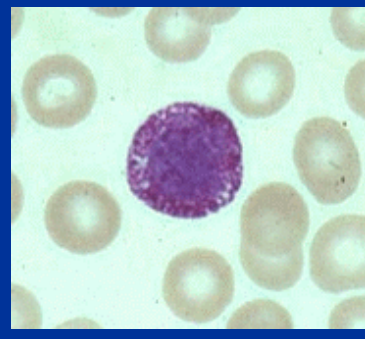
Basophils and Mast cells



- Similar to each other, but functional differences exist in responses of basophils, & serosal and connective tissue mast cells

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Actions of Mast cells (and basophils)

- triggered by allergens, drugs, C3a & C5a, cationic peptides, injury, ...
- primary mediators, in granules immediate reactions,
  - edema, mucus, bronchial constriction, recruitment of granulocytes.
- newly synthesized secondary mediators from mast cells and infiltrating granulocytes, and direct effects of the granulocytes (neutrophils and eosinophils) constitute the “late phase reaction” in 2-3 hours.

80

Lipid Mediators from Mast Cells

PAF platelet activating factor: aggregates platelets, histamine release, bronchial spasm, vascular permeability, vascular dilatation, chemotactic for eosinophils and neutrophils

Arachidonic acid products

- lipoxygenase pathway
  - LTC4, LTD4: potent vasoactive and spasmogenic
  - LTB4: chemotactic for granulocytes and macrophage
- cyclooxygenase pathway
  - PD2: vasoactive, mucus production

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Non lipid Mediators from Mast Cells

- **Biogenic amines:**
  - histamine: bronchial spasm (smooth muscle contraction), vascular permeability, mucus secretion
  - adenosine: bronchial spasm, decreased platelet aggregation
- **Chemotaxins/cell activators**:
  - eosinophil chemotactic factor, neutrophil chemotactic factor
- **Enzymes**
  - proteases and acid hydrolases that activate kinins and C'
- **Cytokines**
  - **TNF-alpha**, chemotactic for neutrophils and eosinophils; IL1,3,4,5,6, gm-csf, chemokines

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Molecules involved in Vasodilation

- smooth muscle relaxes
  - rapid: histamine, serotonin;
  - slower: nitric oxide, kallikrein, PGE2, PGI2, PGD2 (mast cells)
- opening of precapillary arteriole sphincters causes more flow and pooling of blood (hyperemia) in post capillary venules (creates flare on skin)
  - Prostaglandins, histamine
- (veinules relax as well)

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Molecules involved in Vascular Permeability and Inflammatory Exudate

- activation/retraction of endothelium leads to increased vascular permeability
  - –histamine (and serotonin), PAF, bradykinin, LTC4, LTD4, LTE4: often begins in 10-15 minutes
- extravascular protein-rich exudate osmotically draws water
  - –includes fibrinogen (clotting and opsonin), globulins (antibodies), albumen
  - –fluid accumulation produces edema (and cutaneous wheal).
  - –edema (extravascular interstitial fluid) (or effusion in cavities) with cutaneous wheal

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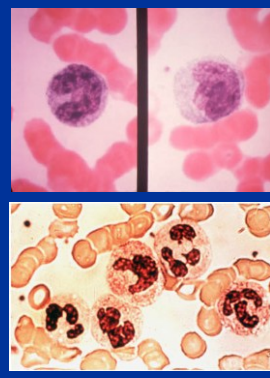
Vascular changes alter blood cell movement



- Flow slows and stasis develops
  - Instead of "trains" of wbc leading rbc, axial flow develops with a plasmatic zone
  - activated leukocytes marginate and adhere to endothelium
  - smaller vessels become packed with rbc ("stasis")
- capillary fluid is lost, plasma becomes, high in protein (albumen and fibrinogen), viscous, increases erythrocytes' tendency to form rouleaux (coin-like stacks)
  - reduced dielectric constant due to increased albumin and fibrinogen (asymmetrically charged) coating
  - cells associate

85

Leucocytes Migration

Margination

Adhesion Expression of Adhesion molecules

- endotelium: Selection (stimolated by IL-1, TNF)

- leucocytes : integrins

Migration

- (first 6 – 24 h : neutrophiles
- 24 – 48 h : monocites)

CHEMOTAXIS : movement along a chemical gradient (chemical mediators)

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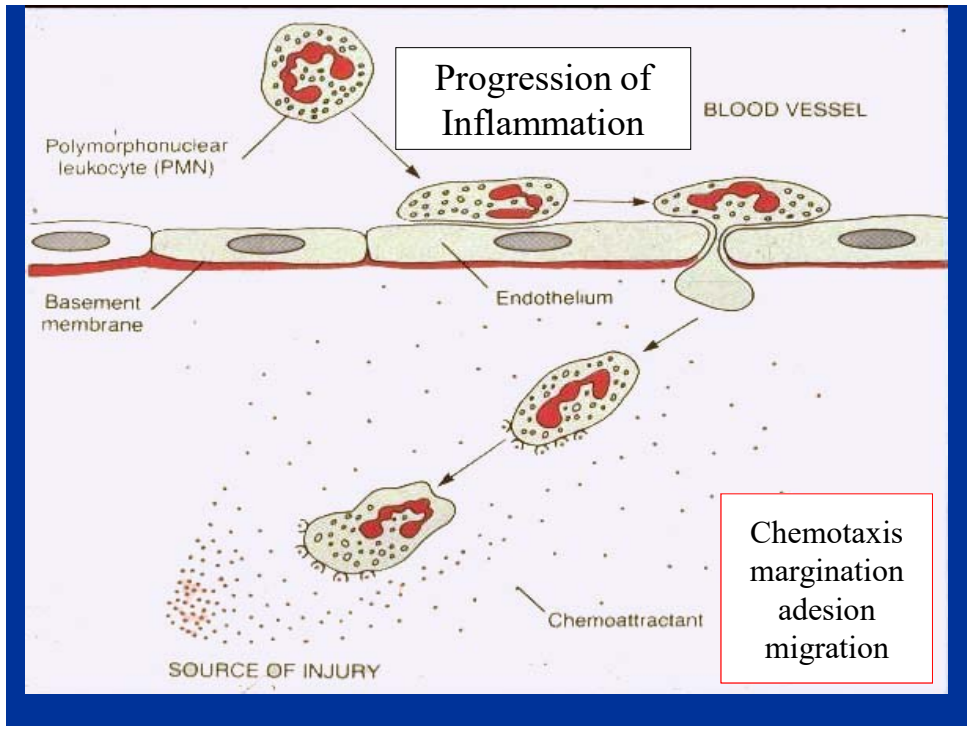
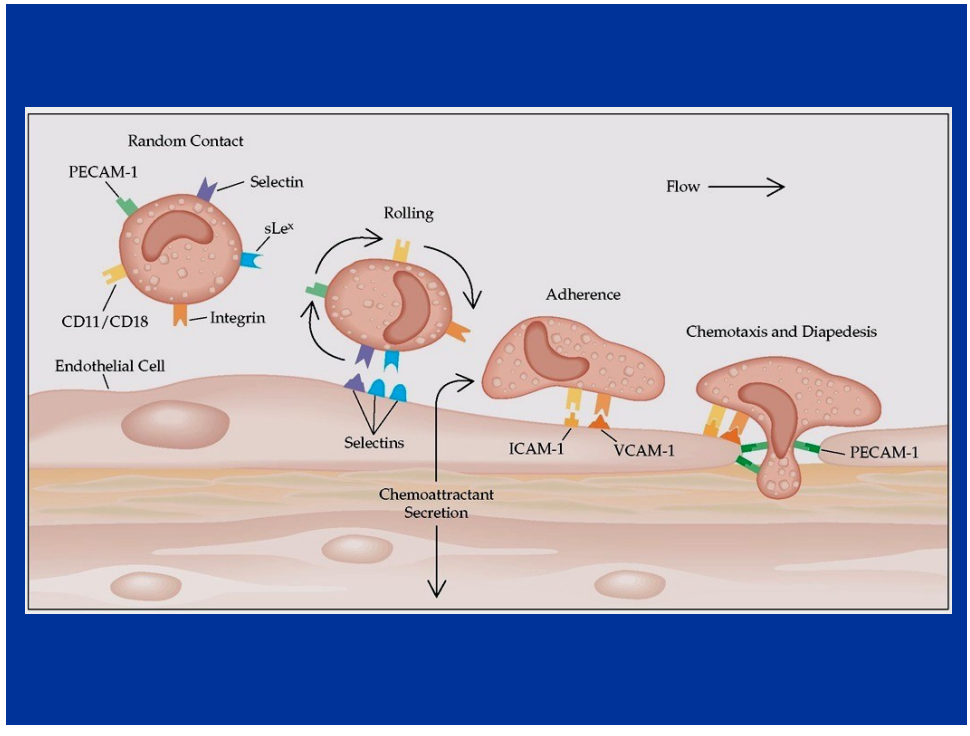

Progression of Inflammation
BLOOD VESSEL
Polymorphonuclear leukocyte (PMN)
Basement membrane
Endothelium
Chemoattractant
SOURCE OF INJURY
Chemotaxis
margination
adesion
migration

87


Random Contact
PECAM-1
Selectin
sLe^x
CD11/CD18
Integrin
Endothelial Cell
Rolling
Selectins
Flow
Adherence
ICAM-1
VCAM-1
Chemotaxis and Diapedesis
PECAM-1
Chemoattractant Secretion

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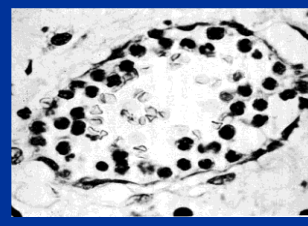
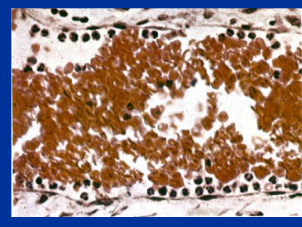
Margination and Pavementing





- leukocytes localize outside the axial flow and become activated express cell adhesion molecules
- marginating neutrophils adhere due to increased contact and sticking to endothelial selectins on activated endothelium
- pavementing over an endothelial gap (neutrophil integrins) leads to extravascularization

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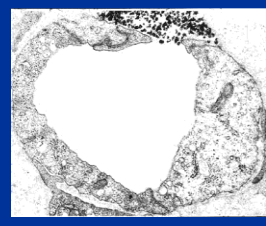
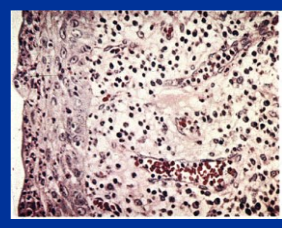
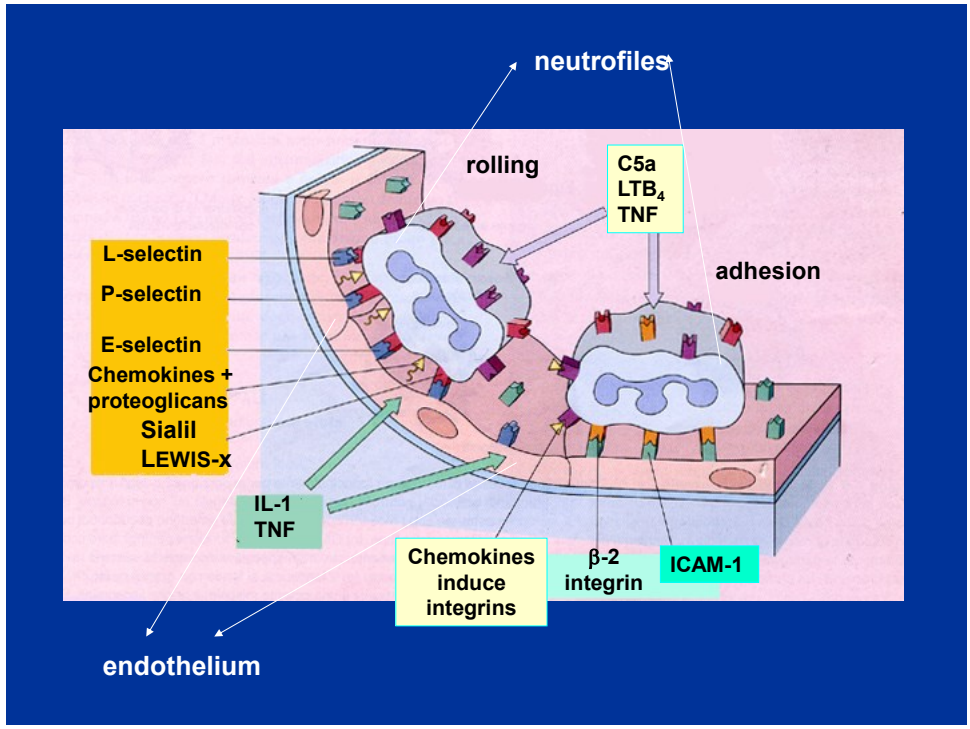
Emigration and Infiltration





- Diapedesis
  - pseudopods lead, dissolving basement membrane and the neutrophils leave the vasculature through open gaps
- Transmigration (emigration)
  - chemotactic signals draw leukocytes into tissues; **haptotaxis**, movement along insoluble gradient, e.g.attractants on ECM

90



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A neutrophil-dominated infiltrate develops

- Mediators initiate neutrophil activation and chemotaxis
- neutrophils become the hallmark cell of the early acute inflammatory infiltrate.
- Neutrophils are attracted mainly by:
  - C5a, LTB4; and later by chemokines, TNF-α
  - Bacterial infections provide n-formyl peptides e.g. FMLP.
- **Neutrophil infiltration** peaks at 6-24 hours, often release contents of lysozomes and secrete lipid mediators, which contribute to the clinical "late phase reaction (4hrs.+)" along with mast cells .

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Products from Neutrophils and Macrophage

- **Eicosanoids**
  - 5-lipoxygenase:LTB4 (chemotactic), LTC4, LTD4, LTE4 (vasoconstriction, bronchospasm, and vascular permeability)
- **from Lysosomes**
  - PLA2, proteases, myeloperoxidase, defensins, cationic proteins

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Products from Activated Macrophage

- **Cytokines**
  - IL-1, IL-6, TNF, chemokines e.g. IL-8
  - activate endothelium, liver (IL-1 and 6, TNF-α), brain (IL-1), IL-8 is chemotactic for neutrophils, and especially TNF, contribute to toxic shock.
- **Other**
  - proteases, hydrolases, coagulation factors, Complement, growth factors (PDGF, EGF,FGF,TGF-beta), eicosanoids, nitric oxide, reactive oxygen metabolites
- **Nitric oxide**
  - from macrophage and endothelium, relaxes smooth muscle (vasodilation), reduces platelet and neutrophil aggregation and adhesion

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Oxygen Derived Products (neutrophils)

- kill microbes, damage endothelial, rbc, and parenchymal cells; inactivate antiproteases (which can lead to destruction of the ECM).
- A respiratory burst of oxygen consumption and HMP shunt support NADPH oxidase generation of superoxide (O2-), which is converted to H2O2 by superoxide dismutase.
- H2O2 reacts with myeloperoxidase plus halide (Chloride) to form hypochlorous acid (HOCl), the major bactericidal agent made by phagocytes (neutrophils, not monocytes).
- Enhanced by Fe2+, H2O2 forms the potent hydroxyl radical (• OH).
- Nitric oxide (NO) reacts with oxidants to form a variety of toxic NO derivatives.
- Oxygen derived radicals are **detoxified** by ceruloplasmin, transferrin, superoxide dismutase, catalase & glutathione peroxidase (H2O2), produced by various cells.

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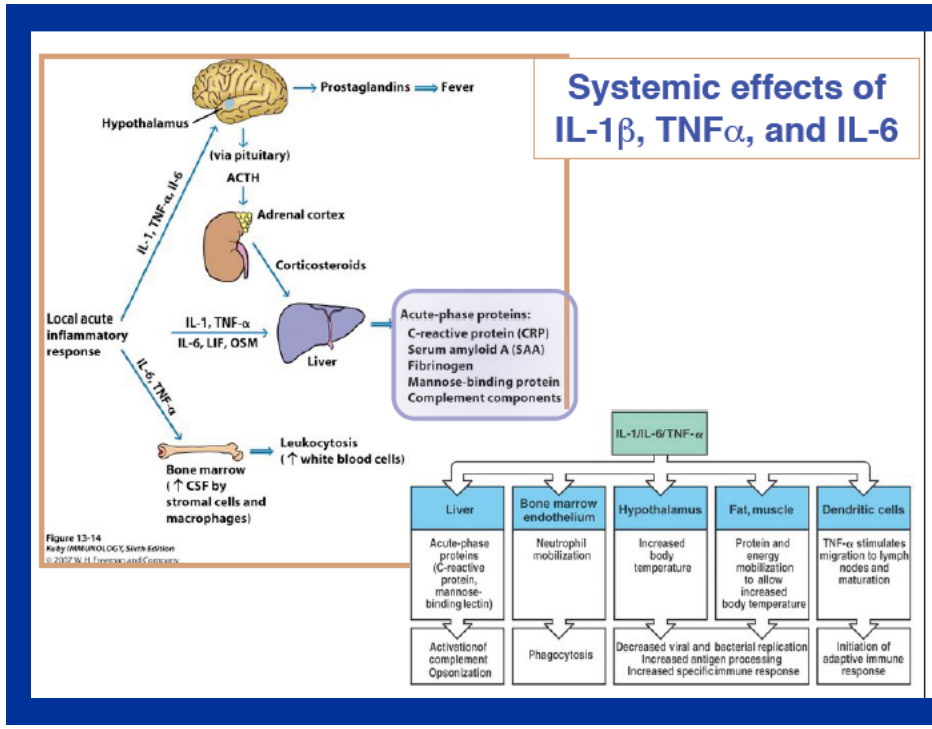

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The acute phase response

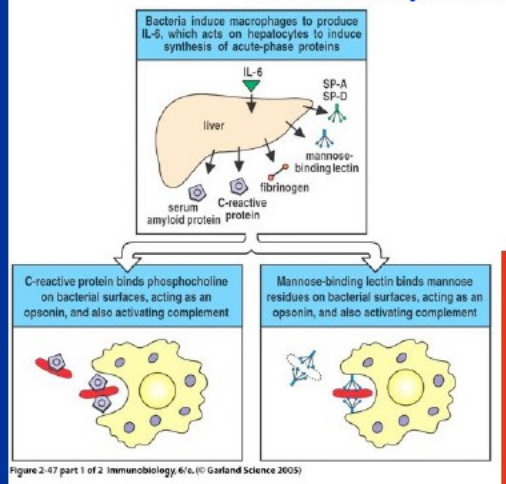
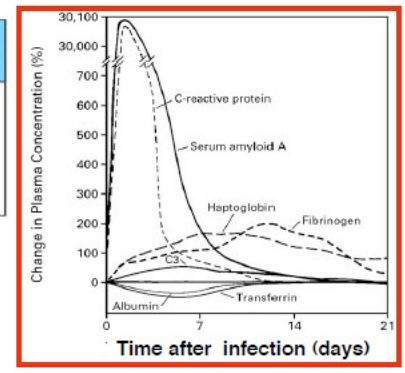
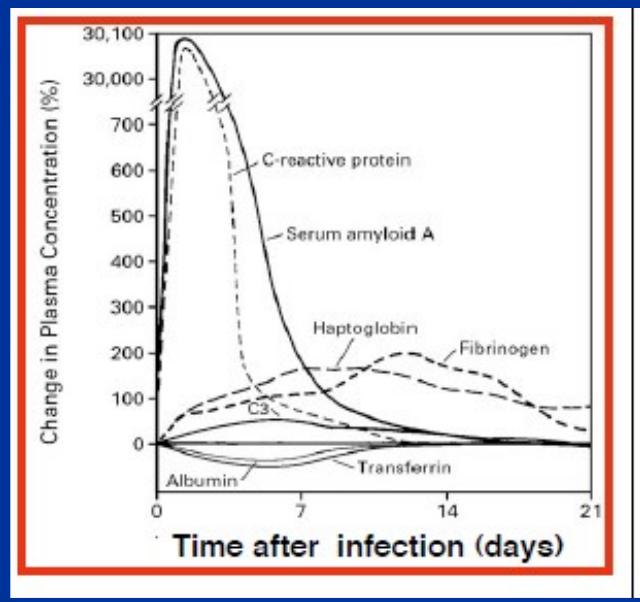


Figure 2-47 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)



97



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What are the "The Five Cardinal Signs of Acute Inflammation" ?

- heat (Calor)
  - dilatation causes hyperemia, which raises extremities to core body temperature
- redness (Rubor)
  - hyperemia pools rbc in capillaries
- pain (Dolor)
  - pressure and mediators (bradykinins, PG--) stimulate nerves
- swelling (Tumor)
  - exudate causes edema in interstitial tissues
- loss of function (Functio Laesa)
  - changes in microenvironment interfere with function

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Systemic Acute Phase Reactions

- IL-1, IL-6, TNF from macrophage and endothelium at higher levels stimulate the liver, brain, muscle; and indirectly other tissues
- sustained increase in CSFs e.g. GM-CSF, G-CSF, M-CSF, FGF stimulates marrow production of new cells
- liver metabolism changes blood protein levels
  - rapid ↑CRP and SAA, SAP
  - slow ↓transthyretin, albumin, transferrin
  - slow ↑ fibrinogen, protease inhibitors
- acute phase proteins e.g. fibrinogen elevate the Eritrocyte Sedimentation Rate
- fibrinogen and Creactive Protein act as opsonins

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