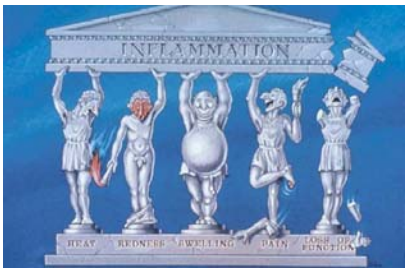
Inflammation

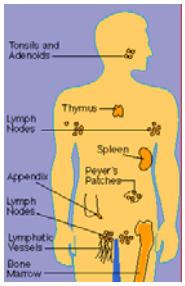
- acute phase reaction, cytokine network, chemokines



1

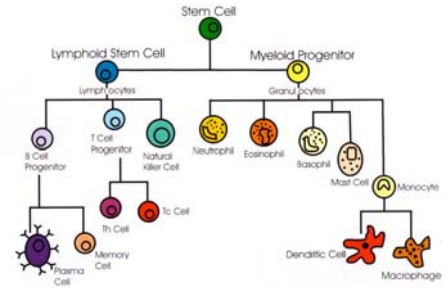
Immune system

- able to recognize "body's own" from "foreign"
  - terms antigen × allergen × superantigen
- main functions
  - self-defence
    - together with stress reaction inflammation represents body response to threat
  - homeostasis
    - continuous removal of old and damaged cells in order to maintain structural and functional integrity
  - immune surveillance on replication and reproduction
    - removal of mutated cells
- organs and tissues of immune system
  - bone marrow and peripheral blood
  - thymus
  - spleen
  - lymphatic nodes
  - extranodal lymphatic tissue (MALT)
    - tonsils, Peyers plaques, ...



2

Immune system cells



3

Mechanisms of immune defence

- according to the way of antigen recognition:
  - non-specific (innate)
  - specific (adaptive)
- according to the participating system:
  - humoral
  - cellular
- other cooperating systems:
  - clotting cascade
  - fibrinolysis
  - vascular endothelium
  - acute phase proteins

	NON-SPECIFIC	SPECIFIC
phylogenically	older	younger
rate of the reaction	minutes	hours – days
immunological memory	no	yes
cells	phagocytes, NK-cells	lymphocytes T and B
humoral factors	complement	antibodies
auxiliary systems	coagulation cascade fibrinolysis, endothelium, platelets, acute phase proteins (liver)	

	HUMORAL	CELLULAR
active mechanisms	complement, antibodies	phagocytes, NK-cells, lymphocytes and their products (cytokines)
defence against	extracell. bacteria toxins, some viruses	viruses, fungi, tumours, intracell. bacteria

4

Reactions of immune system

- physiological = inflammation as a defence phenomenon
  - acute inflammation
    - reactions of vascularized tissues to pathogenic stimulus – material released from damaged or dead cells due to physical or chemical injury, or infection, .. – with aim to remove to restore the integrity
- pathological = inflammation as a auto-aggressive phenomenon
  - chronic inflammation
    - inadequately intense or repeated exposure to pathologic stimulus or inability of normal reaction due to **immunodeficiency**
  - inflammation as a result of immunopathologic reaction
    - **allergy (atopy)**
    - **autoimunity**
  - rejection of transplanted tissue

5

Physiologic immune reaction

- aiming to eliminate foreign material from organism
- 3 phases:
  - antigen recognition
  - amplification of signal
  - effector phase (together with feedback regulation)
    - in some cases inflammation consist almost exclusively non-specific immune reactions (non-infectious etiology)
    - in case of antigenic stimulus specific immune reaction follows
      - ☛ it depends on particular antigen whether reaction will be predominantly humoral or cellular
      - ☛ non-specific immune reactions are largely responsible for clinical picture of any inflammation!
- extent of damaged cells/amount of antigen, way of entering the body, time of exposure and general condition of organism govern the intensity of reaction
  - inapparent
  - local
    - calor, rubor, dolor, tumor, functio laesa
  - systemic
    - fever, tachycardia, hyperventilation, prostration, loss of appetite, metabolic and endocrine alterations

6

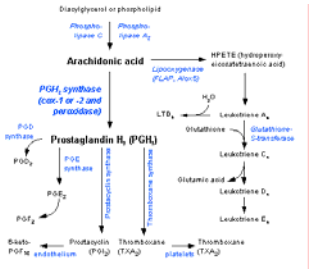
Innate immunity – acute inflammation

- participating cells/proteins
  - endothelium
  - thrombocytes
  - coagulation cascade + fibrinolysis
  - polymorphonuclears (PMN, neutrophil granulocytes)
  - complement
  - mast cells, basophils, eosinophils
  - monocytes/macrophages
- feedback regulation
  - inhibitors of complement intermediate products
  - anti-proteases (α1-antitrypsin, α2-macroglobulin)
  - antioxidant enzymes (SOD, catalase, ..)
  - anti-inflammatory cytokines
  - fibrinolysis

7

Endothelium and platelets

- endothelium
  - ↑ formation of NO by inducible NOS
    - reaction of NO with superoxide produces aggressive peroxynitrite
  - formation of PGI_2 by PLA_2 and PLC from arachidonic acid (membrane phospholipids)
  - increased expression of adhesive molecules (E-selectin, integrins, ICAM, VCAM, PECAM)
    - at first, they allow "rolling" of PMN on the endothelium
    - later, firm adhesion and diapedesis into extravascular tissue (extravasation)
  - formation of anti-aggregative and fibrinolytic factors
    - tPA, thrombomodulin
  - formation of pro-aggregative factors
    - endothelin, PAF, vWf
- thrombocytes
  - formation of TXA_2, serotonin, PAF, PAI, PDGF



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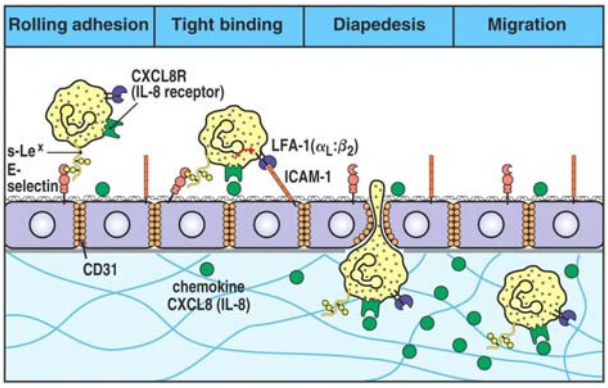
Activation of endothelium



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PMN

- number increases (leukocytosis)
- diapedesis into tissues
- antigen recognition does not require HLA
  - phagocytosis → metabolic "burst" (production of ROS, RNS etc.)
    - superoxide produced by NAD(P)H oxidase → (SOD) peroxide → hydrochlorous acid (myeloperoxidase)
    - superoxide (NAD(P)H oxidase) → peroxide (SOD) → hydroxyl radical (Fenton reaction with Fe)
  - secretion of proteolytic lysosomal enzymes
  - activation of PLC a PLA_2 → PGI_2, PGE_2, TXA_2, LT
  - PMN produce cytokines
    - IL (1, 6, 8), TNFα, G-CSF, GM-CSF, interferon, PAF, plasminogen activator, LTA, ...

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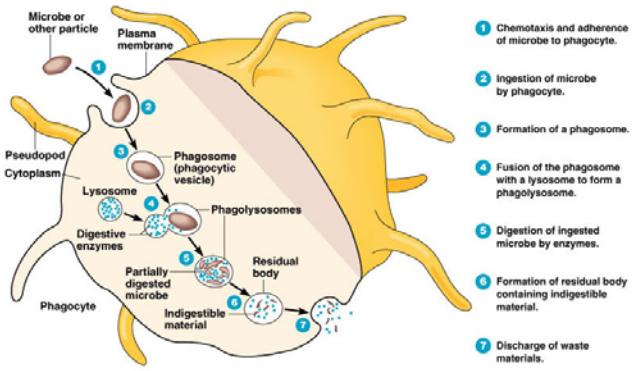
Phagocytosis by PMN



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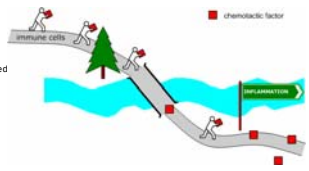
Chemotaxis and its mediators

- Chemotaxis
  - directed movement of cells in the concentration gradient of soluble substances (chemotactic factors, chemotaxins or chemoattractants)
    - positive – along the concentration gradient
    - negative – against the concentration gradient
  - biologic activity mediated by specific cell surface receptors for these factors, their expression is modulated by cytokines
  - other factors participate in chemotaxis too - extracellular matrix, adhesion molecules, cytoskeleton and some LMW substances
- Origin of chemotactic factors
  - degranulating cells
  - activated immune cells
  - activated endothelial cells
- Types of chemotactic factors
  - oligopeptides of bacterial origin
  - cytokines (chemokines, interleukins, TNFα, IFNγ) produced by PMN and macrophages
  - complement cascade product C5a
  - kallikrein and bradykinin from coagulation cascade
  - AA derivatives - prostaglandins, leucotriens (mainly from macrophages)
  - tachykinins (substance P, neurokinin A, substance K, neuropeptid K, neuropeptid γ, neurokinin B)
  - VPF (vascular permeability factor)
- Functions:
  - chemotaxis – inflammation, immune reactions, wound healing
  - activators of many types of immune effector cells
  - increase of vascular wall permeability
  - some of them vasodilatation, bronchoconstriction, or increase of mucus production



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Monocytes/macrophages, NK-cells

- phagocytosis without previous contact with antigen
- production of cytokines
- macrophages function as antigen presenting cells (APC)
  - transition between non-specific and specific immunity

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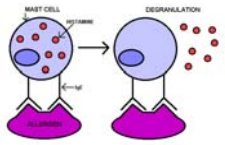
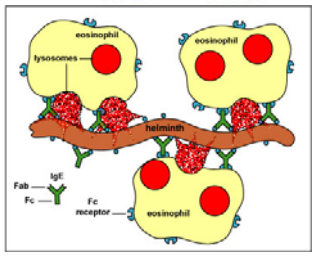
Mast cells (basophils), eosinophils

- mast cell (basophils)
  - localised mainly perivascularly in the skin and mucous membranes
  - following stimulation by antibodies (IgE) or complement products they release content of their granules:
    - histamin
    - serotonin
    - heparin
    - proteolytic enzymes
    - derivatives of arachidonic acid
    - cytokines



- eosinophils
  - active mainly in parasitic infections and allergies
  - limited ability to phagocytose
  - release of granules – cationic proteins - eosinophil peroxidase (EPO), major basic protein (MPO), eosinophil-derived neurotoxin (EDN)
    - binding to the neg. surfaces – damage, increased permeability (e.g. pulmonary edema in ARDS)



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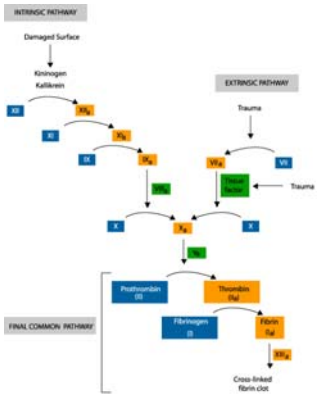
Coagulation cascade



- both types of activation active in inflammation
- ↑ kallikrein → bradykinin → vasodilation

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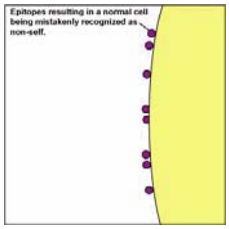
Complement system (CS)

- biochemical cascade of more that 35 proteins (directly active or regulatory) leading to:
  - cytolysis
  - chemotaxis (mainly C5a)
  - opsonization ("marking" the pathogens for phagocytosis), mainly C3b
  - anaphylatoxins (mainly C3a) – activation of mast cells
- 3 pathways of activation of CS:
  - classical
  - alternative
  - lectin pathway
    - at the beginning of inflammation CS is first activated by alternative pathway
    - then, when antibodies are formed, by classical pathway



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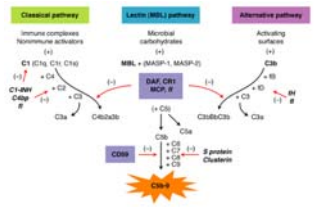
Activation of CS



- all 3 pathways lead to the formation of C3-convertase, which begins to form membrane attack pathway (incl. C5b, C6, C7, C8 and polymeric C9) leading to the formation of MAC (membrane attack complex)
  - MAC is a cytotoxic end-product of CS producing trans-membrane channel causing osmotic lysis of target cell
- **classical pathways** begins with activation of complex C1 by binding of C1q on antigen-antibody complex
  - C1 cleaves C2 and C4 to C2b and C4b, they bind together to form C3-convertase
- **alternative pathway** begins with hydrolysis of C3 on the surface of patogen
  - C3 cleaves to C3a and C3b
  - some C3b bind to pathogen (factor B); this complex is then cleaved by factor D to Ba and C3-convertase Bb
- **lectin pathway** is homological to classical one, but there is an opsonin mannan-binding lectin (MBL) instead of C1q
  - this pathway is activated by binding of MBL on mannose residues on the surface of pathogen; this activates serin proteases associated with MBL (MBL-associated serine proteases MASP-1 and MASP-2)
  - MASP cleave C4 and C2 to C4b and C2b, thus producing C3-convertaseas in the classical pathway

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Adaptive immunity

- APC (macrophages, dendritic cells, ..)
- regulatory lymphocytes T (Th, CD4+)
- effector lymphocytes T (cytotoxic Tc, CD8+) and B (plasmocytes)
- antibodies
- cytokines
  - interleukins/TNF
  - interferons
  - chemokines
  - growth factors
  - colony-stimulating factors

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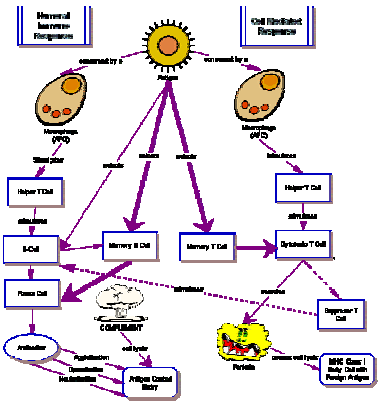
Specific immune reactions



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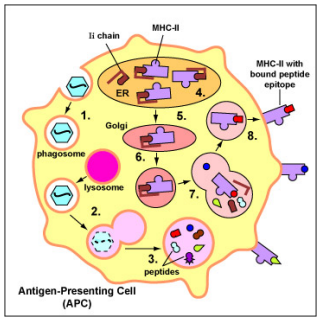
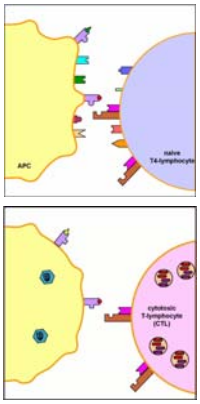
APC – Th / APC-Tc cooperation





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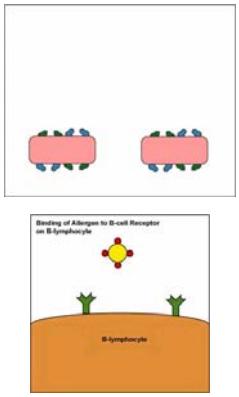
Activation of B-lymphocytes



- contact with antigen + stimulation by cytokines from Th
  - proliferation and differentiation into plasmocytes
  - production of antibodies

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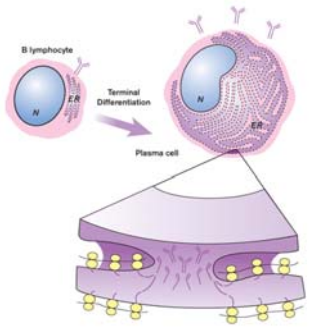
Antibodies



- classes
  - IgM, IgA, IgD, IgE, IgG
- function
  - neutralisation of pathogens
  - activation of CS
  - antibody dependant cell-mediated cytotoxicity (ADCC)
    - NK cells attach to Fc fragments → perforins & granzymes → caspases → apoptosis

22

Cytokines

- Variable group of soluble proteins and peptides
  - act in pM to nM concentrations
- Cytokines regulate function of almost all immune cells under physiologic as well as pathologic conditions
  - cell division - mitogens or anti-mitogens (growth factors)
  - "survival" or "suicide" factors (apoptosis)
  - transformation and differentiation factors
- Some cytokines soluble while others membrane-bound
  - balance between soluble and bound fraction is a regulated parameter
- Most of them pleiotropic, i.e. different biological activities
- Important modulators during embryogenesis and organogenesis
- Nomenclature (often reflects first described function or origin):
  - interleukins, lymphokines, monokines, interferons, TNF, CSF etc.
- Cytokine receptors
  - tyrosine kinase receptors with intrinsic kinase activity
    - IL-6, EGF, PDGF, CSF-1 etc.
  - serine kinase receptors with intrinsic kinase activity
    - TGF-β
  - receptors without intrinsic kinase activity
  - G-protein coupled receptors and ion-gated receptors
- Cytokines act via their receptors, however, not directly, but via activation of specific transcription factors
  - expression of immediate early response genes
  - their products regulate transcription of delayed early response genes

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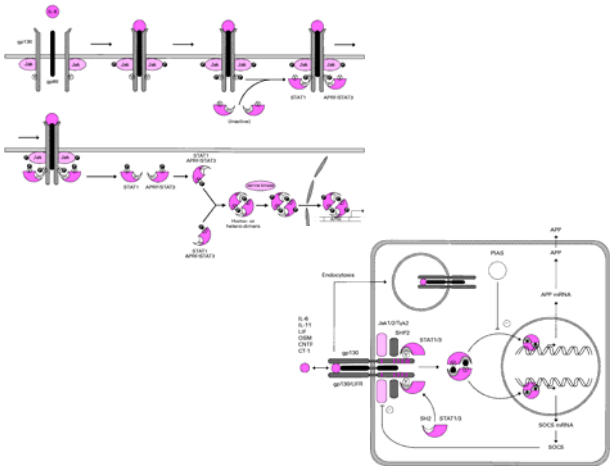

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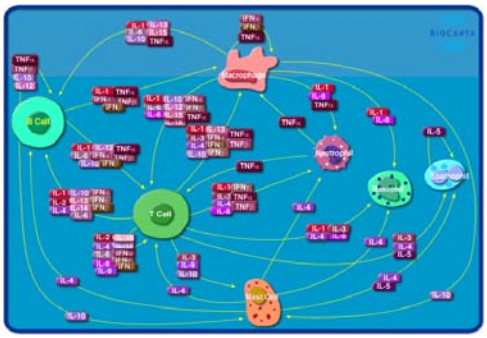
Cytokine network



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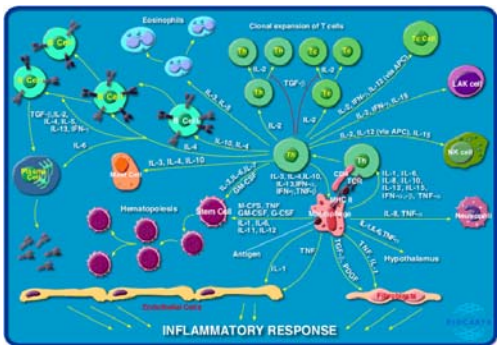
Summary - inflammation



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Th1/Th2 cytokines

- Th1 and Th2 class cytokines are produced by different sub-populations of CD4+ Th-lymphocytes
  - Th1 cytokines favour cell-mediated immune responses
    - IL-2, IFNγ, IL-18, TNFβ, etc.
  - Th2 cytokines favour differentiation of B-cells and humoral immunity
    - IL-4, -5, -6, -10, -13, etc.
- Imbalance between the 2 sub-populations pathogenic factor in allergic vs. autoimmune diseases

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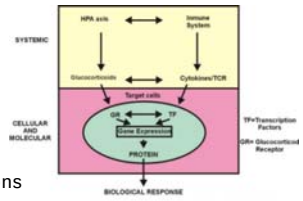
Systemic manifestation of inflammation

- increase of body temperature – fever
- leukocytosis
- tachycardia
- hyperventilation
- fatigue
- loss of appetite
- metabolic and endocrine alterations
  - ↑ gluconeogenesis, protein catabolism, ACTH, cortisol, glucagon, T4, aldosterone, vasopressin, Cu
  - ↓ albumin, Fe, Zn, transferin
- synthesis od reactants of acute phase



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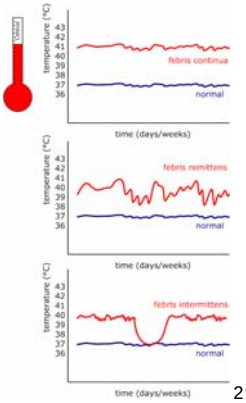
Fever (pyrexia)

- Temperature is regulated in the hypothalamus
- Pyrrogens
  - exogenous – lipopolysacharide (LPS) of gramm-negative bacteria
  - endogenous
    - cytokines IL-1, -6, TNFα produced by phagocytes
    - necrotic debris
- Pathophysiology
  - LPS binds to circulating LPS-binding protein (LBP)
  - complex LPS-LBP binds to CD14 of macrophages and induce production and release of endogenous pyrogens
  - in the brain – paraventricular area of hypothalamus – binding to the cytokine receptors → activation of PLA_2, COX-2 and PGE_2 synthase in AA pathway → production of PGE_2 = an ultimate febrile mediator → re-setting the thermoregulatir to higher set-point
  - brain orchestrates heat-producing mechanisms
    - heat production by increased muscle tome (shivering thermogenesis)
    - endocrine response (epinephrine and thyroid hormones) → higher metabolic rate
    - prevention of heat loss by peripheral vasoconstriction
    - non-shivering thermogenesis in adipose tissue???????
- Stages of fever
  - stadium incrementi – until the reaching the new temperature set-point
  - stadium acme – keeping the temperature, stop of shivering and vasoconstriction
  - stadium decrementi – decrease of the temperature after the decrease of pyrogenic stimulation
    - fact (crisis) – with profuse perspiration
    - slow (lysis)
- Pyrexia can be classified as
  - low-grade: 38 - 39 °C (99.5 - 102.2 °F)
  - moderate: 39 - 40 °C (102.2 - 104 °F)
  - high-grade: > 40 °C (> 104 °F)
  - hyperpyrexia: > 42 °C (> 107.6 °F)
- Several typical types of fever
  - febris continua (daily fluctuation <1°C)
  - febris remittens (daily fluctuation >1°C)
  - febris intermitens (periods of normal and increased temperature)
- Physiological significance of fever
  - unclear, growth of some microbes might be slowed-down by higher temperature, higher metabolic rate might help to immune and circulatory system work more effectively
  - too high fever is harmful



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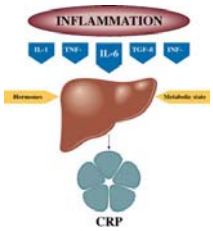
Reactants of acute phase (RAF)

- heterogenous group of plasma proteins synthesized in liver in response to inflammatory stimuli
  - this is a systemic response to local injury mediated by cytokines (IL-1, IL-6, IL-11, INFγ and TNF-α) produced by PMN and macrophages
- variable function of RAF
  - C-reactive protein (CRP)
    - CRP (pentamer) cleaved by PMN enzymes to produce opsonization and chemotactic factors
  - serum amyloid A protein (SAA)
  - orosomucoid
  - inhibitors of proteases
    - α1-antitrypsin, α1-antichymotrypsin, α2-macroglobulin
  - some coagulation factors
    - fibrinogen, prothrombin, fVIII, plasminogen
  - transport proteins
    - haptoglobin, hemopexin, feritin
  - complement system factors
    - C3



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Dynamics of RAF

Class	RAF	Increase
Inhibitors of proteases	α_1-antitrypsin α_1-antichymotrypsin	4 x 6 x
Coagulation proteins	fibrinogen prothrombin factor VIII plasminogen	8 x
Complement system factors	C1s C2b C3, C4, C5 C9 C5b	2 x
Transport proteins	haptoglobin hemopexin feritin	8 x 2 x 4 x
Scavenger proteins	ceruloplasmin	4 x
Others	α_1-acid glykoprotein (orosomucoid) SAA protein CRP	4 x 1000 x 1000 x

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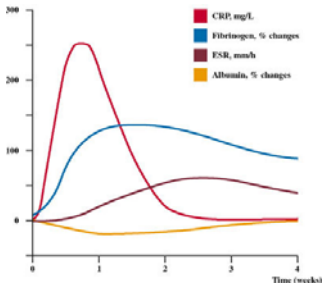
Typical changes of plasma levels of CRP, fibrinogen, ESR (erythrocyte sedimentation rate) and albumin during acute inflammation



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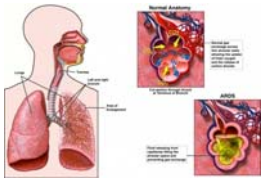
Critical situations connected to systemic inflammation

- Sepsis
  - widespread activation of immune and coagulation systems due to septicaemia
  - sepsis can progress to septic shock and multi-organ dysfunction
- Disseminated intravascular coagulation (DIC)
  - generalised activation of clotting cascade by various stimuli incl. infection leading to the multiple thrombi (early phase) and then hypo-coagulative state (late phase)
- Adult respiratory distress syndrome (ARDS)
  - life-threatening condition causing lung edema and fluid transudation into the air sacs due to increased permeability of the pulmonary microcirculation
    - they can be damaged by released proteolytic enzymes and other mediators of inflammation
  - fluid inhibits gas exchange between the air and the bloodstream



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