Inflammation
- acute phase reaction, cytokine network, chemokines

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, ...

Immune system cells

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

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

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

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
Innate immunity – acute inflammation

- participating cells/proteins
  - endothelium
  - thrombocytes
  - coagulation cascade (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

Endothelium and platelets

- endothelium
  - formation of NO by inducible NOS
  - reaction of NO with superoxide produces aggressive peroxynitrite
- formation of PGI2 by PLA2 and PLC from arachidonic acid (membrane phospholipids)
- increased expression of adhesive molecules (E-selectins, integrins, ICAM, VCAM, PECAM)
  - at first, they allow "rolling" of PMN on the endothelium
  - later, firm adhesion and extravasation into extravascular tissue (extravasation)
- formation of anti-aggregative and fibrinolytic factors
  - tPA, thrombomodulin
- formation of pro-aggregative factors
  - endothelin, PAF, vWF

Activation of endothelium

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 PLAs → PGI2, PGE2, TXA2, LT
  - PMN produce cytokines
    - IL (1, 6, 8), TNFα, G-CSF, GM-CSF, interferon, PAF, plasminogen activator, LTA, ...

Phagocytosis by PMN

Chemotaxis and its mediators

- Chemotaxis
  - directed movement of cells in the concentration gradient of soluble substances (chemokinetic factors, chemoattractants or chemotactic factors)
  - 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 immune effectors, and external factors
  - 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 products (C5a)
    - kallikrein and bradykinin from coagulation cascade
    - AA derivatives - prostaglandins, leucotriens (mainly from macrophages)
    - tachykinins (substance P, neurokinin A, substance K, neuropeptide K, neuropeptide γ, neurokinin B)
  - Functions (vascular permeability factor)
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

Mast cells (basophils), eosinophils

- mast cell (basophil) - localised mainly perivascularly in the skin and mucous membranes
  - following stimulation by antibodies (IgE) or complement C5b-9, they release content of their granules:
    - histamin
    - serotonin
    - heparin
    - proteolytic enzymes
    - derivatives of arachidonic acid
    - cytokines
- eosinophils
  - active mainly in parasitic infections and allergies
  - 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)

Coagulation cascade

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

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

Activation of CS

- all 3 pathways lead to the formation of C3-convertase, which begins to form membrane attack pathway (MAC, 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 begin with activation of complete C3 by binding of C1q on antigen-antibody complex
  - C1q binds to C1r and C1s, then C1r and C1s cleave C4 and C2 to C4b and C2b, they bind together to form C3-convertase
- alternative pathway begins with hydrolysis of C3 on the surface of pathogen
  - C3 cleaves to C3a and C3b
  - C3b binds to factor B; this complex is then cleaved by factor D to Bb and C3-convertase
- lectin pathway is homologous to classical one, but there’s an opsonin mannose-binding lectin (MBL) instead of C1q

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
Specific immune reactions

- Contact with antigen + stimulation by cytokines from Th
  - Proliferation and differentiation into plasmocytes
  - Production of antibodies

APC – Th / APC-Tc cooperation

Activation of B-lymphocytes

- Contact with antigen + stimulation by cytokines from Th
  - Proliferation and differentiation into plasmocytes
  - Production of antibodies

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

Cytokines

- Variable group of soluble proteins and peptides
  - Act in pH 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
  - Serine kinase receptors with 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
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

Fever (pyrexia)

- Temperature is regulated in the hypothalamus
- Pyrogens:
  - Cytokines (IL-1, IFNγ, TNF) produced by phagocytes
  - LPS
- Pathophysiology:
  - LPS binds to circulating LBP
  - LBP + LPS complex binds to CD14
  - Activation of PLA2, COX-2, PGE2 synthase
  - Production of PGE2
  - Re-set the temperature set-point
  - Brain orchestrates heat-producing mechanisms
    - Muscle tone (shivering)
    - Non-shivering thermogenesis

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, transferrin
- Synthesis of reactants of acute phase

Reactants of acute phase (RAF)

- Heterogenous group of plasma proteins synthesized in liver in response to inflammatory stimuli
  - C-reactive protein (CRP)
    - Cleaved by PMN enzymes
  - Serum amyloid A protein (SAA)
  - α1-antitrypsin, α1-antichymotrypsin, α2-macroglobulin
  - Fibrinogen, prothrombin, FVIII, plasminogen
  - Haptoglobin, hemopexin, ferritin
  - Complement system factors
- Variable function of RAF
  - Optimize opsonization and chemotactic factors
  - Serum amyloid A protein (SAA)
  - Inhibitors of proteases
  - α1-antitrypsin, α1-antichymotrypsin, α2-macroglobulin
  - Coagulation factors
  - Hepatoplasmin, transferrin, ferritin

Cytokine network

Summary - inflammation
**Dynamics of RAF**

<table>
<thead>
<tr>
<th>Class of Proteins</th>
<th>RAF</th>
<th>Increase</th>
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<tr>
<td>Inhibitors of proteases</td>
<td>α₁-antitrypsin, α₁-antichymotrypsin</td>
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<tr>
<td>Coagulation proteins</td>
<td>fibrinogen, erythromycin, factor VIII, plasminogen</td>
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<tr>
<td>Complement system factors</td>
<td>C1q, C2b, C3, C4, C5, C9, C1q</td>
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<td>Transport proteins</td>
<td>haptoglobin, hemopexin, ferritin</td>
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<td>Scavenger proteins</td>
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<tr>
<td>Others</td>
<td>α₁-acid glycoprotein (orosomucoid)</td>
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<tr>
<td></td>
<td>SAA protein</td>
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<tr>
<td></td>
<td>CRP</td>
<td>1000 x</td>
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</table>

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

**Critical situations connected to systemic inflammation**

- **Sepsis**
  - widespread activation of immune and coagulation systems due to sepsis and multi-organ dysfunction
- **Disseminated intravascular coagulation (DIC)**
  - generalized activation of clotting cascade by various stimuli incl. infection leading to 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