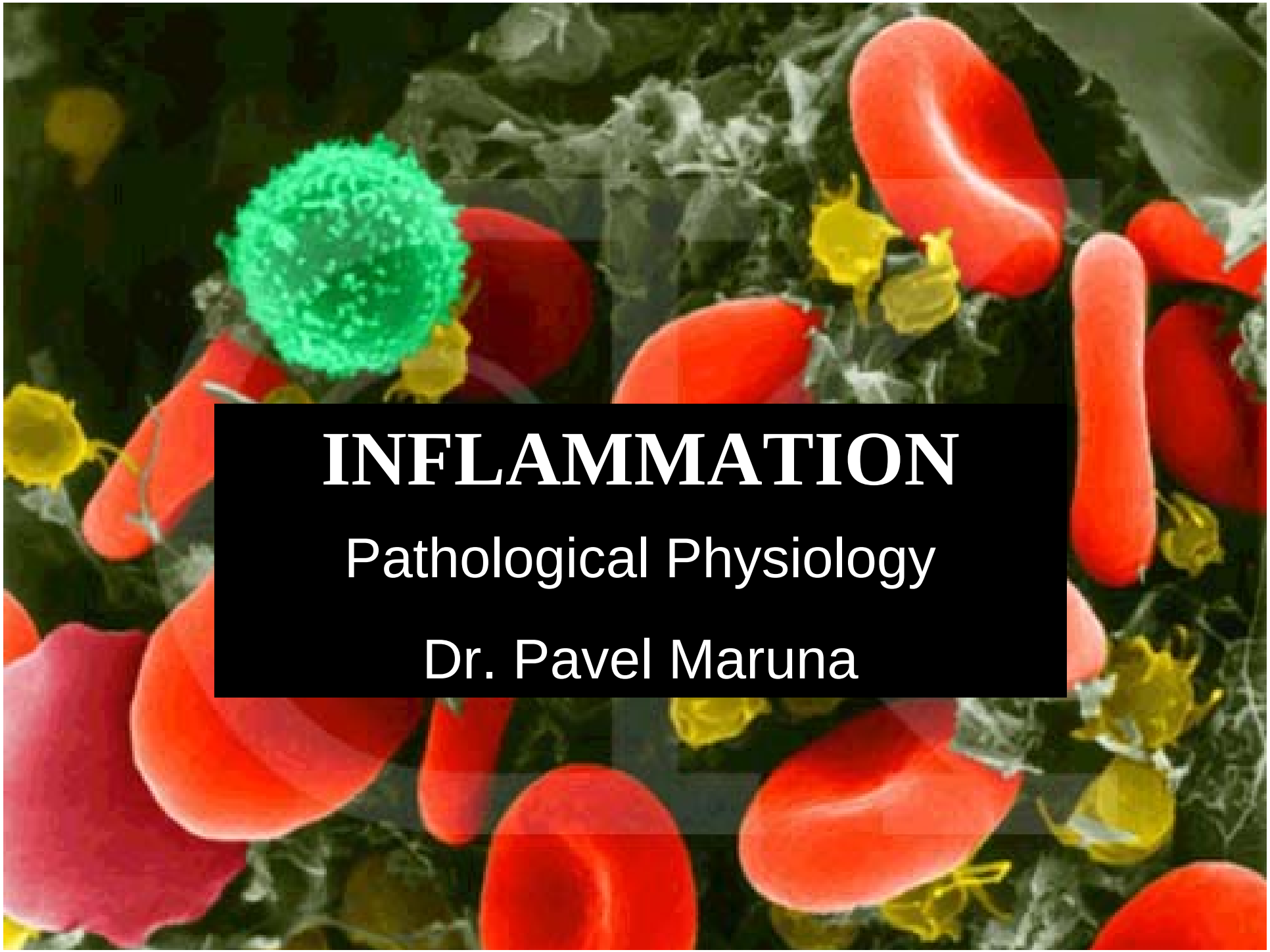


INFLAMMATION

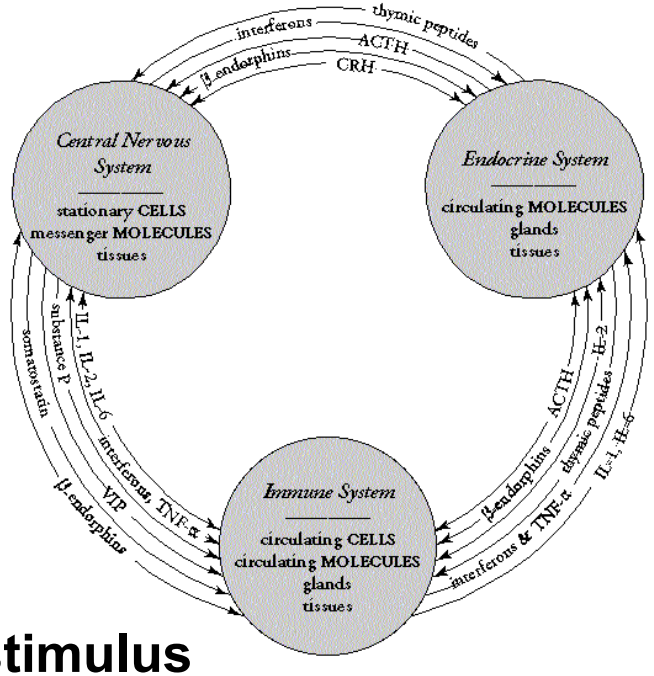
Pathological Physiology

Dr. Pavel Maruna

Universal defense systems

1. Stress reaction (common adaptive syndrome)

- Generalized reaction
- Activation and coordination from CNS
- Universal (non-specific) character



2. Inflammation

- Primarily local reaction ($\rightarrow$ generalization)
- Reaction of vascularized tissues
- Specific response – targeting against initial stimulus

Definition

= The complex system of defense reactions of vascularized tissues against pathogenic stimulus (insult) of different character

The aim of inflammation is:

- **elimination of a cause,**
- **removal of an irreversibly damaged tissue,**
- **consecutive tissue regeneration or reparation, restoration of impaired both metabolism and function of organs, the return to dynamic balance status**

Including other defense actions: coagulation, regeneration, tissue reparation, neurohumoral responses

Insult (pathogenic factor)

- biological (microbes, parasites)
- physical (trauma, irradiation)
- chemical (toxins, drugs)
- metabolic (hypoxia, hyperglycaemia, metabolic disorders)
- immunological (autoimmune disorders)
- neurohumoral (e. g. stress gastric ulcer)

Defense x Autoaggressive reaction

- depends on results of inflammatory process

The main factor of a defense course of inflammation ...
localization (limitation) and regulation of immune response

x Dysregulation and delocalization

→ Autoaggressive development

→ Organ dysfunction

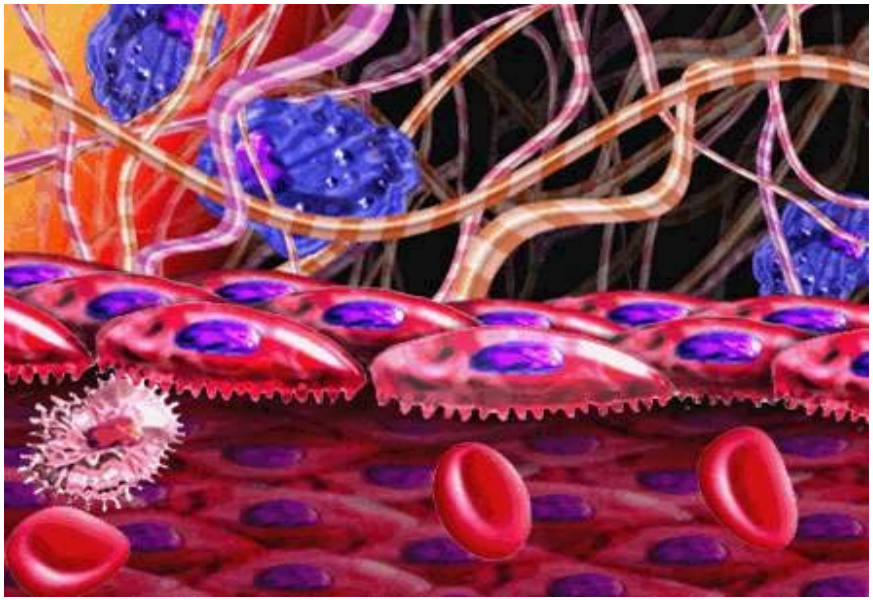
Many inflammatory mediators (cytokines) reveal an ”toxic potential”

- nonspecific (reactive O2 forms, proteolytic enzymes)
- specific (cytotoxicity)

Inflammation local x systemic

Local inflammation – a range and duration of inflam. reaction are limited

Systemic inflammation – non-limited, excessive reaction



Inflammation local x systemic

Local inflammatory response

Symptoms of the local inflammation

- rubor (color)
- calor (temperature)
- tumor (edema)
- dolor (pain)
- functio laesa (dysfunction)

Inflammation local x systemic

Systemic inflammatory response

- Systemic insult leads to systemic inflammatory response.
- Systemic inflammatory response may not be necessarily autoaggressive.
- Inflammatory processes are delocalized, if dysregulation is than added – auto aggressive inflammation starts.

Systemic inflammatory response syndrome (SIRS)

Characteristics:

- Delocalized and dysregulated inflammation process of high intensity. It leads to disorders of microcirculation, organ perfusion and finally to secondary organ dysfunction.
- This secondary dysfunction is **not due to primary insult**, but due to autoaggressive systemic inflammatory response of the organism to the primary insult.
- This systemic inflammatory response syndrome (SIRS), leads without therapeutic intervention to multiple organ dysfunction syndrome (MODS) and death.

Systemic inflammatory response syndrome (SIRS)

Definition:
The presence of 2 or more following criteria

Symptoms	Assessed factors
Body temperature	>38oC or <36oC
Pulse rate	>90 /min
Rate of breathing or PCO2 (arterial blood)	Frequency of breathing >20 /min $PaCO_2$ <32 mm Hg
White blood count or I/T ratio	>12 000/mm^3 or <4 000/m^3 >10%

Systemic inflammatory response syndrome (SIRS)

Generalized reaction to systemic insult (infection, trauma, radiation)

Systemic release of early pro-inflammatory cytokines, their endocrine activities.

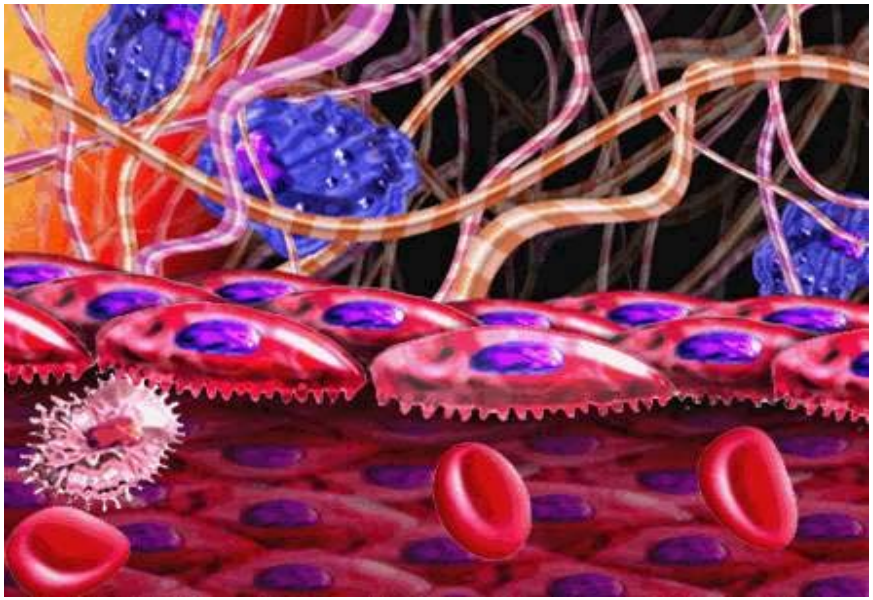
Proinflammatory activity TNF, IL-1, IL-6, IFN-γ ... main source – activated mononuclear cells.

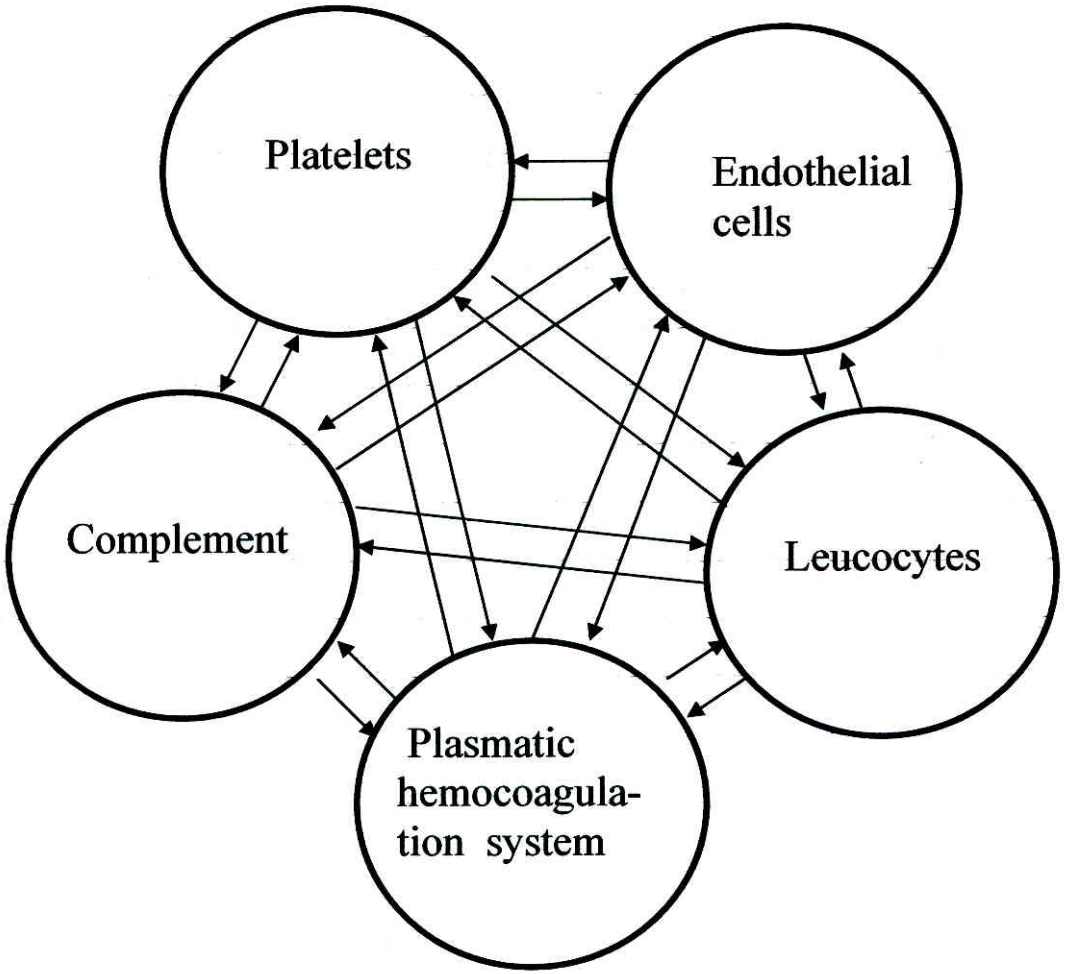
Contraregulatory activity (CARS) of glucocorticoids (cortisol) and anti-inflamm. cytokines (IL-4, IL-10).

Inflammation local x systemic

Mechanisms of an inflammatory responses

- **Endothelium**
- **Blood cells (platelets, leukocytes)**
- **Local humoral factors (plasma coagulation system, complement)**
- **Neuro-endocrine systems**





Systems responsible for inflammatory reactions

Cooperation of the most important inflammatory response systems.

Endothelium

Key regulator of local / systemic response,

An essential role in defense reaction against pathogens, in perfusion regulation

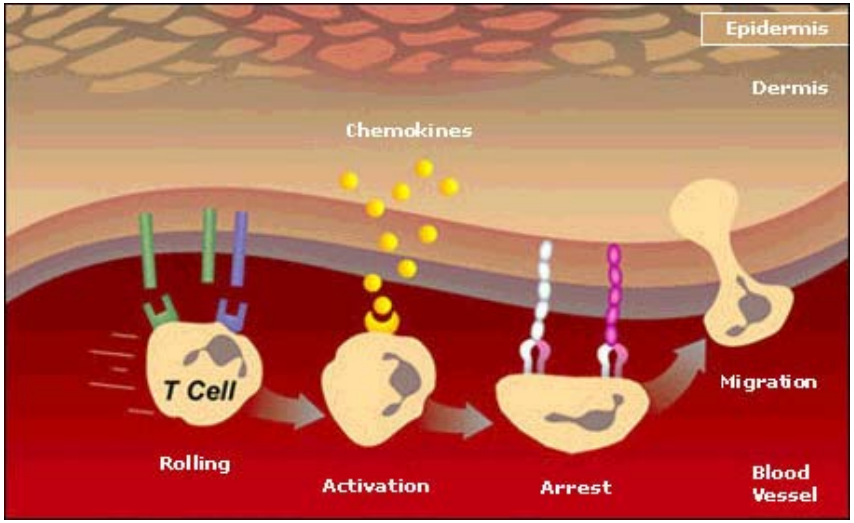
Key functions:

- **Anti-thrombogenic potential of blood vessel wall (thrombomodulin, PG I_2)**
- **Local regulation of vessel tone (NO, PG I_2 ... $\rightarrow$ smooth muscle of arterioles and venuls)**
- **Regulation role in inflammation (directly by insult or indirectly via inflamm. mediators)**

Endothelium

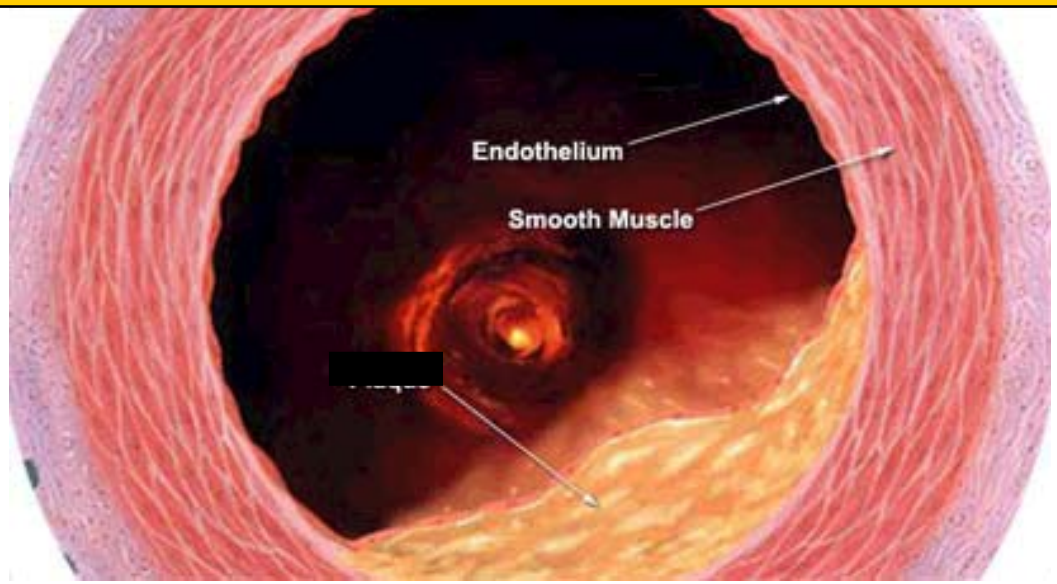
Regulatory role during inflammation:

- **Vasodilation (NO)**
- **Cytokine activation and production**
- **↑ permeability (→ penetration of proteins, antibodies)**
- **Receptor-coordinated migration of leukocytes to perivascular space**
- **Expression of adhesive molecules**
- **Pro-thrombogenic reaction**



Endothelium

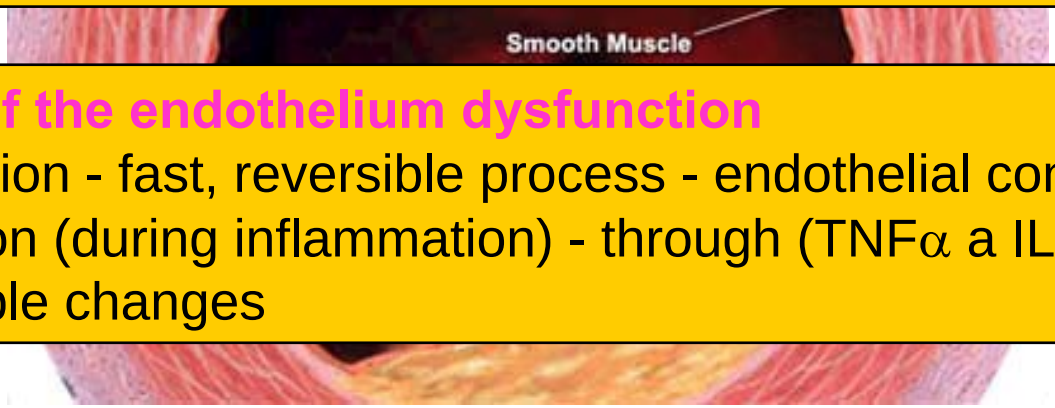
Pathogenetic role of endothelium in septico-toxic shock



Endothelium

Vasodilatatory and antithrombotic mediators
- NO
- prostacycline (PGI2)

Vasoconstrictive and prothrombotic mediators
- endothelin-1
- thromboxan A2



Levels of the endothelium dysfunction
- Stimulation - fast, reversible process - endothelial contraction
- Activation (during inflammation) - through (TNFα a IL-1β), irreversible changes

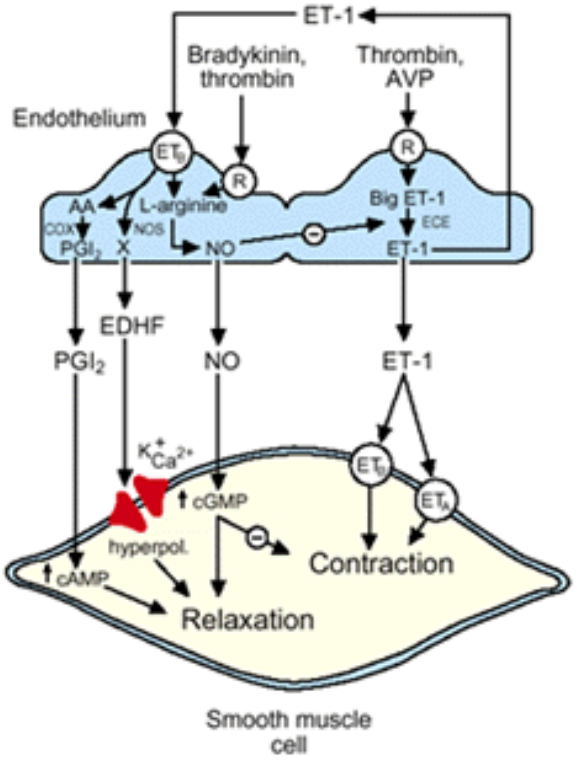
Endothelium

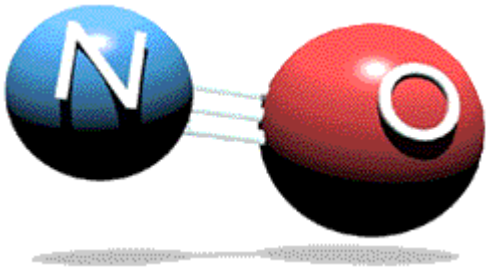
Vasodilatory mediators

- anti-thrombotic function
- anti-adhesive, anti-aggregation effect to platelets and leukocytes
- „protective“ role in relation to endothelium

Vasoconstrictory mediators

- pro-thrombotic function
- pro-aggregattion, pro-adhesive effects to platelets and leukocytes
- “aggresive” role in endothelial dysfunction





Endothelium

Nitric oxide

Reactive radical $NO^{\cdot}$

Product of NO-synthase: L-arginin → L-citruline + NO

Non-receptor activity, short half-life, local effects

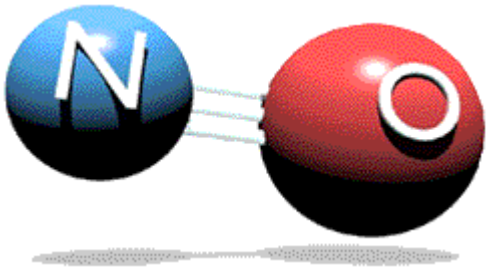
$NO^{\cdot}$ acts in smooth muscle cells to activate soluble guanylate cyclase

Activation of soluble guanylate cyclase → cGMP → ↓ cytoplasmic Ca^{2+} → relaxation

NO-synthase isoenzymes:

Constitutive NO-synthase (endothelium, neurons)

Inducible NO-syntáza (endothelium, leukocytes) - after cytokine stimulation (IFN-γ, IL-1, IL-6, TNFα), LPS or PAF



Endothelium

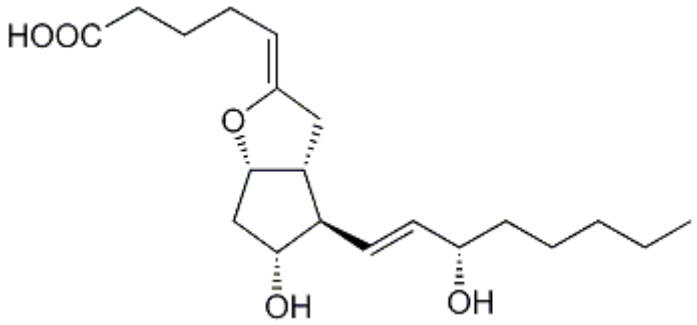
Nitric oxide

$NO^{\cdot}$ - strong cytotoxic effect depends on reaction with superoxide radical with production of peroxynitrite $ONOO^-$. End-products are OH- radical and nitric acid:

$$NO^{\cdot} + \text{superoxide} \rightarrow \text{peroxynitrit } ONOO^- \rightarrow \ OH^- + HNO_3$$

Dysregulated systemic inflammation - autoaggressive NO overproduction:

- **cytotoxic effect**
- **systemic vasoparalysis, life-threatening and often refractory to therapy**



Endothelium

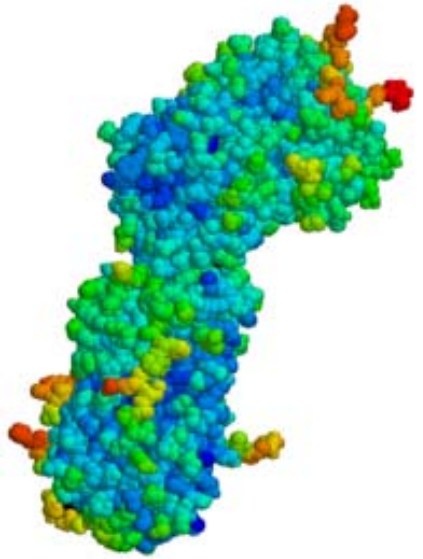
PGI2

- important vasodilator and anti-thrombotic activity
- produced in endothelial cell membrane from arachidonic acid (= a product of membrane phospholipids)
- a key role of cyclooxigenase in PGI2 origin (pharmacological inhibition by ASA)

TxA_2

- platelets
- antagonism of PGI_2 effects (prothromb., proagrreg., proadhesive)
- potentially destructive effects

Endothelium



Antithrombin

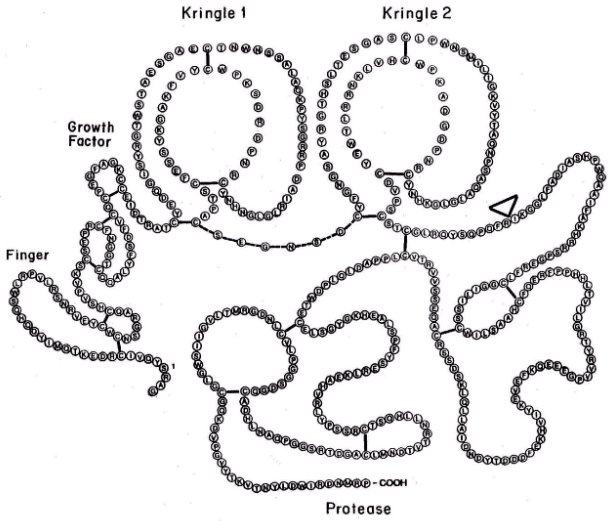
Plasma protein

Products of hepatocytes and endothelium

SERPIN (serine protease inhibitor).

Decrease bellow 70% normal plasma levels - risk of TEN

Antithrombin interacts with specific membrane-bound receptor to stimulate PGI2 synthesis



Endothelium

Tissue plasminogen activator (tPA)

Activator of fibrinolysis

tPA is regulated via PAI (plasminogen activator inhibitor) in circulation

PAI ... acute phase protein, produced by both leukocytes and endothelium

Endothelium

Endothelin 1

↑↑ **vasoconstrictory activity (ET : NE ... 1:700)**

Angiotensin converting enzyme (ACE)

Membrane bound enzyme (pulmonary, cardiac vascular endothelium)

Vasoconstrictory potential (conversion Ang I → Ang II) + inhibition of bradykinin

Endothelium

Coagulation and endothelium

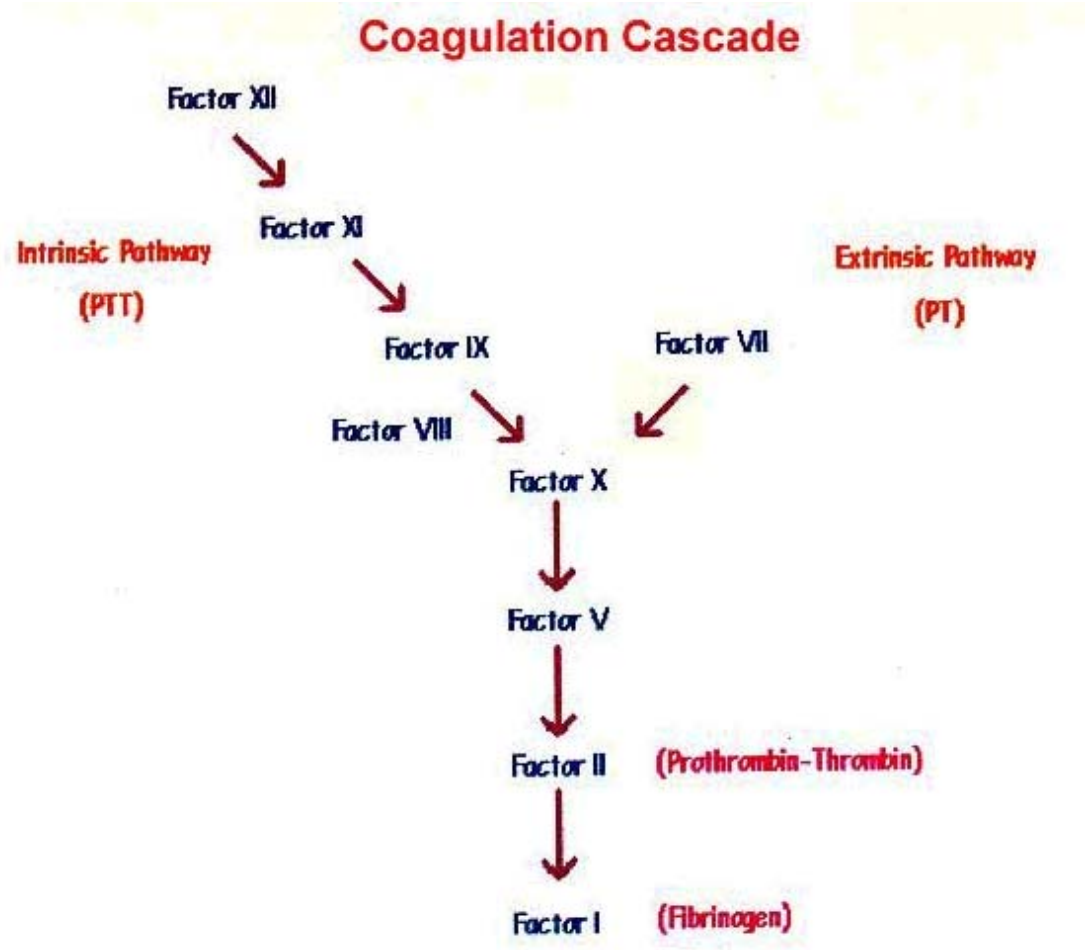
1. Expression of negative charged membrane phospholipids ... matrix for activation of plasma coagulation system

2. The release of von Willebrand factor (vWf) ... adhesion of platelets +activation of f VIII

3. ↑ intercellular permeability ... penetration of f III for coagulation activation

Endothelium

Coagulation and endothelium



Endothelium

Coagulation and endothelium

4. ↑ expression of f III ← cytokine activation (TNF-a, IL-1)

5. Trombomodulin = integral membrane protein of endothelium

Thrombomodulin in complex with thrombin → inactivation of f II (↓ affinity to Fbg, f V, VIII, XIII, platelets x activation of protein C)
APC + protein S → protelytic inactivation of f Va a VIIIa

Endothelium

Coagulation and endothelium

6. Heparan sulphate proteoglycans ... anticoagulant activity

- specific binding of plasma antithrombin (as cofactor)
→ induction of conformation changes of antithrombin
→ inhibition of thrombin
- stimulation of TFPI release from endothelium

Endothelium

Adhesive receptors

- a key role in cell-cell interactions and cell-matrix interaction
- expression on endothelial cells, Leu, platelets

Selectins

P-selectin (platelets) –adhesion of Neu + Mo to activated Plt and endothelium.
E-selectin (endothelium) – participation on adhesion of Neu, Mo and memory T-cells to endothelium
L-selectin (leukocytes) – binding of Leu to endothelium during inflammation.

Ig family receptors (ICAM-1, ICAM-2, VCAM-1 etc.)
→ Fixed adhesion and transmigration of Leu

Integrins

Intensive expression on membrane
binding to endothelial Ig-family receptors

Platelets

Activated platelets

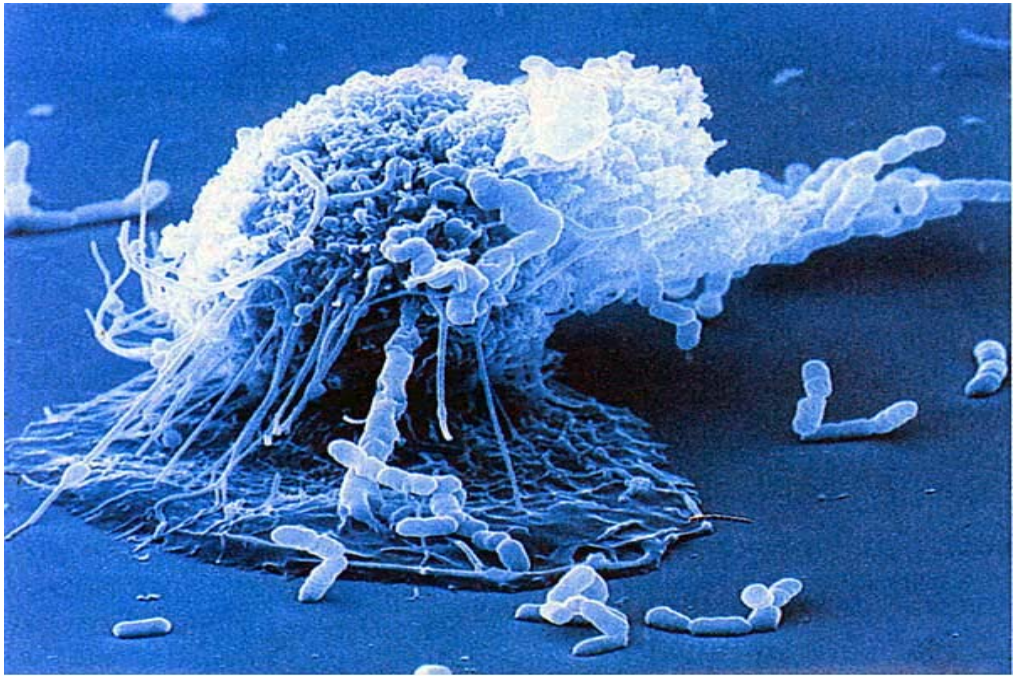
Mononuclear cells

(Monocytes and macrophages)

The main source of inflammatory cytokines

Similar spectrum of mediators as neutrophils - with potential autoaggressive outcome

Main barrier against bacterial, viral or mycotic infection



Mononuclear cells

(Monocytes and macrophages)

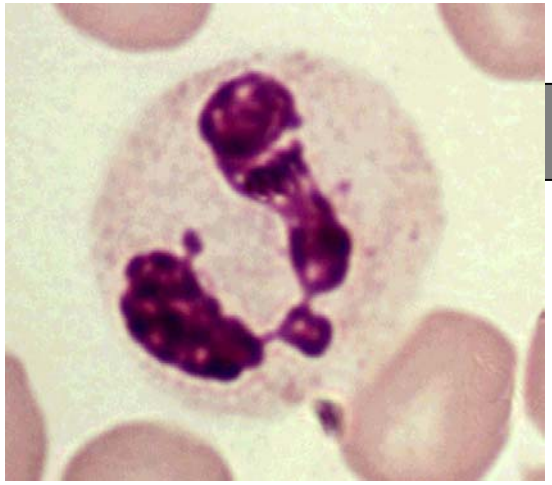
The main source of inflammatory cytokines

Similar spectrum of mediators as neutrophils - with potential autoaggressive outcome

Main barrier against bacterial, viral or mycotic infection

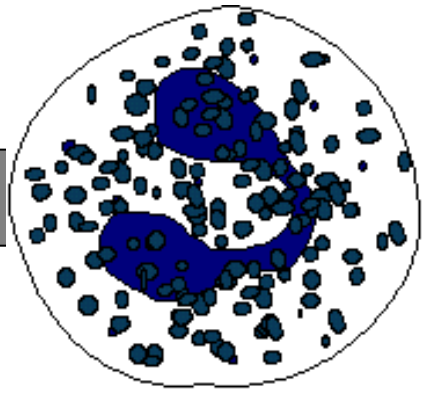
Mononuclear phagocytes
- monocytes of peripheral blood
- tissue macrophages
- both able to perform phagocytosis
- macrophages - main producers of TNF-α a IL-1β

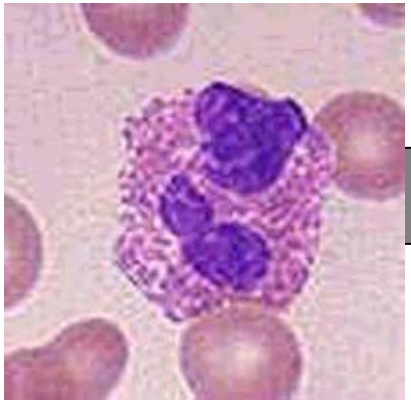
Polymorphonuclear cells



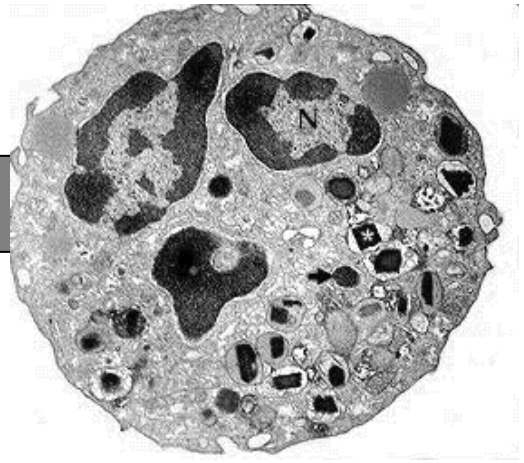
Neutrophils

Basophils

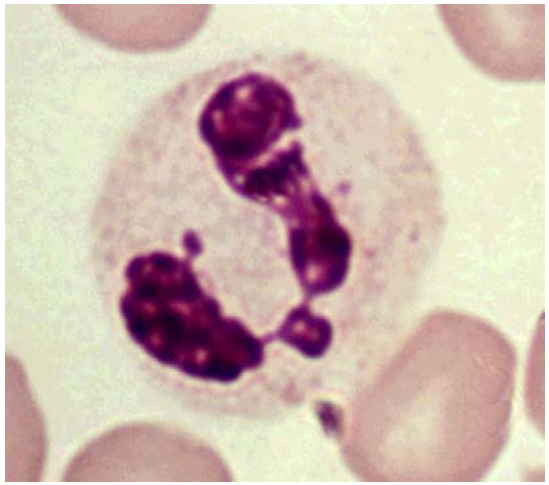




Eosinophils



Polymorphonuclear cells



- concentration in the site of insult
- adhesion to stimulated endothelial cells
- penetration to interstitium

Phagocytosis
Cytotoxic potential
- reactive oxygen intermediates
- hydrolytic enzymes
- antibacterial proteins

Acute phase reaction

= Systemic inflamm. response preserving limited / defense character

Uniform adaptive response to a violation of organism integrity.

Its intensity and duration are limited.

APR is initialized by immune factors – cytokines (TNF, IL-1, and IL-6) and corticoids.

APR includes immune processes, endocrine and metabolic changes, APP synthesis in liver, both water and electrolytic changes, fever etc.

Acute phase reaction

Main influences:

- water, electrolytic and temperature homeostasis
- anti-infectious defense
- modulation of pain
- elimination of irreversible destroyed cells
- sufficient input of energy
- sufficient offer of aminoacids for proteosynthesis
  (antibodies, enzymes, hormones, and for reparation and regeneration.

Sepsis and MODS

Intensive SIRS

= complex dysregulation of homeostasis → destructive disability of organism by its own defense reaction

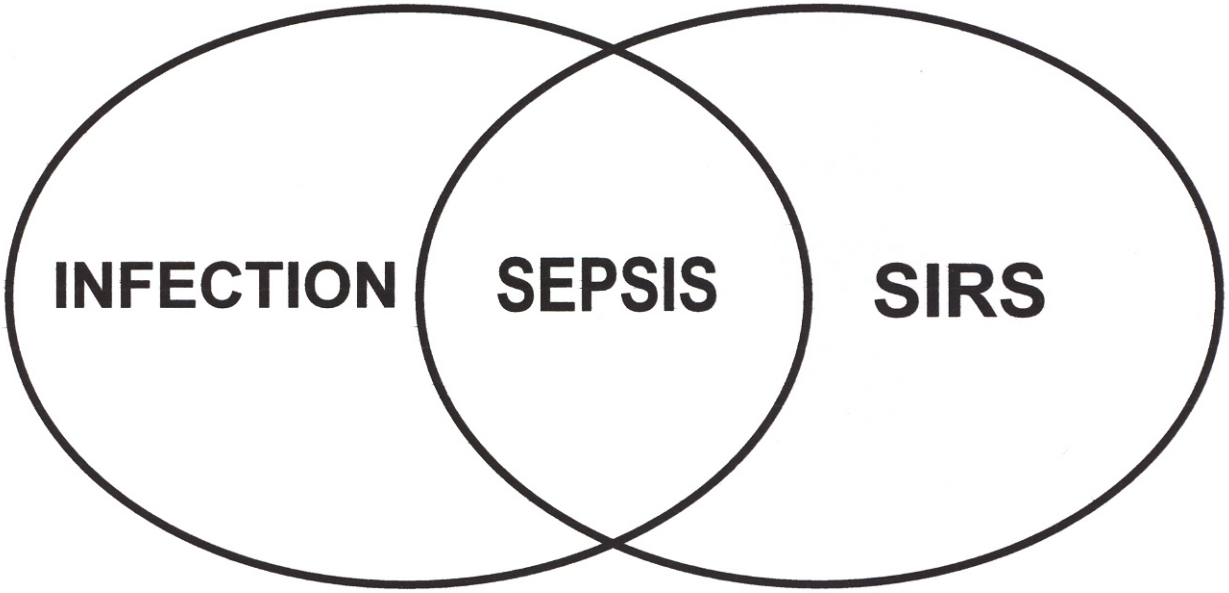
Sepsis

= SIRS with documented infection

Intensive sepsis

= sepsis with manifest cardiovascular alteration

Sepsis and MODS



Sepsis and MODS

Septic shock

MODS (multiorgan distress syndrome)

Hypoperfusion , tissue hypoxia

Reversible → irreversible organ dysfunction

Death of organism

... Auto-aggressive character

Sepsis and MODS

Septic shock

Immunopathology reaction, overexpressed defense reaction

(Bacterial) antigen (LPS)
→ macrophages
→ TNFα, IL-1β
→ endothelium
→ NO
→ vasodilation

hours – days
50 % mortality

Sepsis and MODS

Prognostic factors:

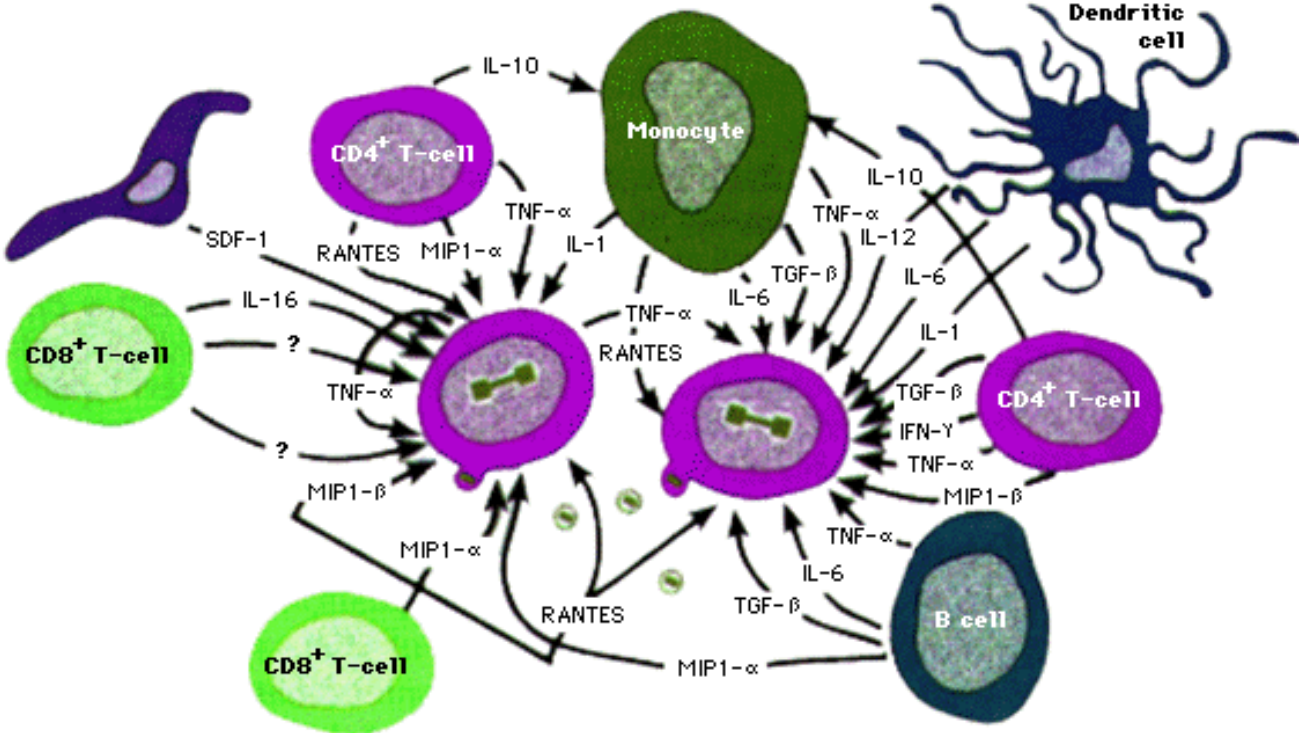
- **intensity and power of initial insult (infection)**
- **interaction of pro / anti- inflamm. factors (cytokines, hormones)**
- **congenital disposition (variability of defense reaction)**
- **iatrogenic factors (corticoids, ATB, imunosupression)**
- **age**

Cytokines

Intercellular communication during inflammatory response is permitted by

1. direct contact cell to cell (membrane receptors, adhesive molecules)

2. soluble mediators (cytokines etc.)



Cytokines

Cytokines = inflammatory mediators

- **Proteins (mainly glycoproteins with low molecular weight)**
- **Products of inflammatory cells (leucocytes, endothelium, platelets), release to intercelular space**
- **Action via specific membrane-bound receptors (Leu, endoth., ...) similarly as hormones**
- **Dominant local effects – autocrine, paracrine (x ... hormones)**
- **Potential systemic activity in an initial phase of inflammation = endocrine effects**

... Systemic inflammatory response

Cytokines

Cytokine network

Interaction of cytokines, hormones, adhesive molecules and coagulation system
The same cytokines are produced from different types of cells (Neu, Mo)
One type of cells can produce different cytokines
Agonistic, antagonistic effects
Negative / positive feedback regulation

Role of corticoids

Cytokines

Interferons (IFN)

Cytokines with antiviral and anti-proliferative potential

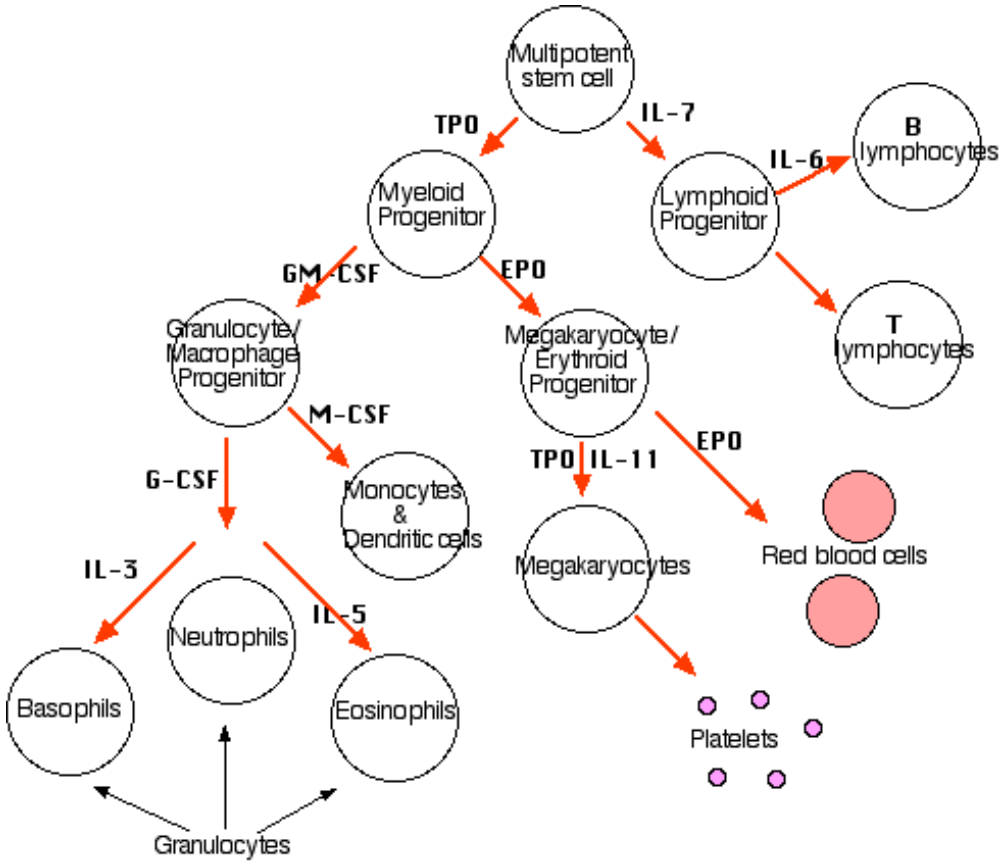
Therapeutic role in some hematological and solid malignancies, polycytaemia vera ...
CML, Grawitz tumor

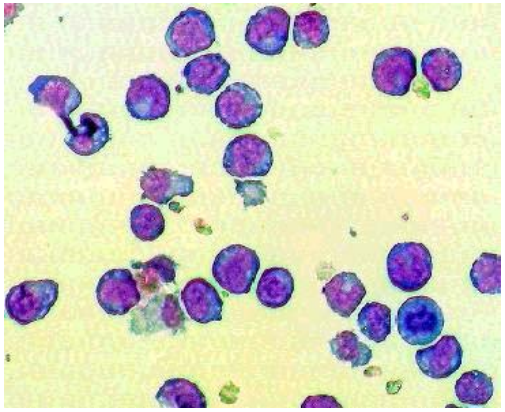
1. Direct anti-proliferate (pro-apoprotic) effect
2. Indirect immunostimulatory effect

Cytokines

Colony stimulating factors (CSF)

Growth factors of hemopoietic stem cells





Therapeutic use for selective stimulation of erythrocytes, platelets, monocytes, granulocytes.

GM-CSF, G-CSF, M-CSF, Epo, Tpo, IL-3, SCF ...

Cytokines

Chemokines (or chemoattractants)

Peptides with chemotactic effects on Gr, Mo and other WBC.

↑ adhesion on endothelium

Cytolytic function

Cytokines

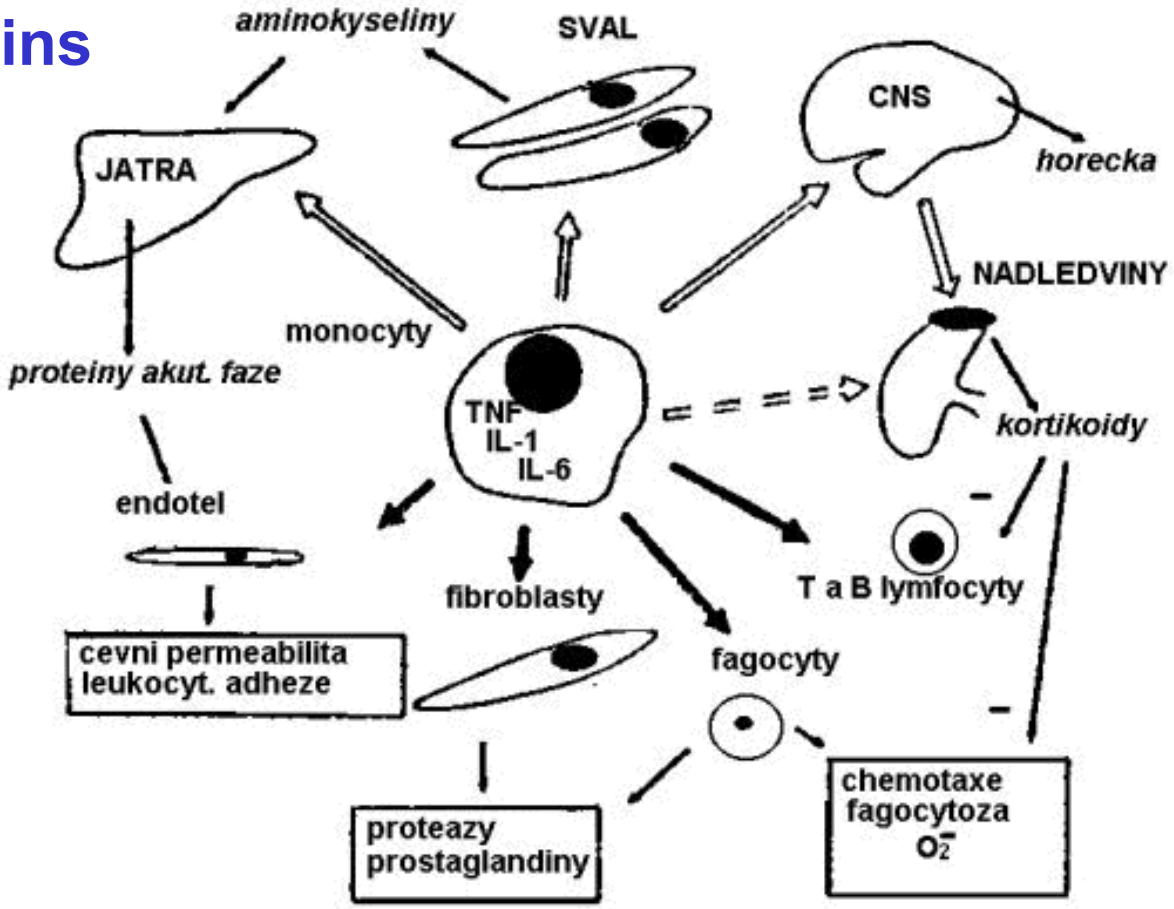
Interleukins (IL)

Inflammatory mediators with pro/anti-inflammatory activity (IL-1, 2, ... 26)

IL-1, IL-6, TNF ... crucial proinflammatory cytokines

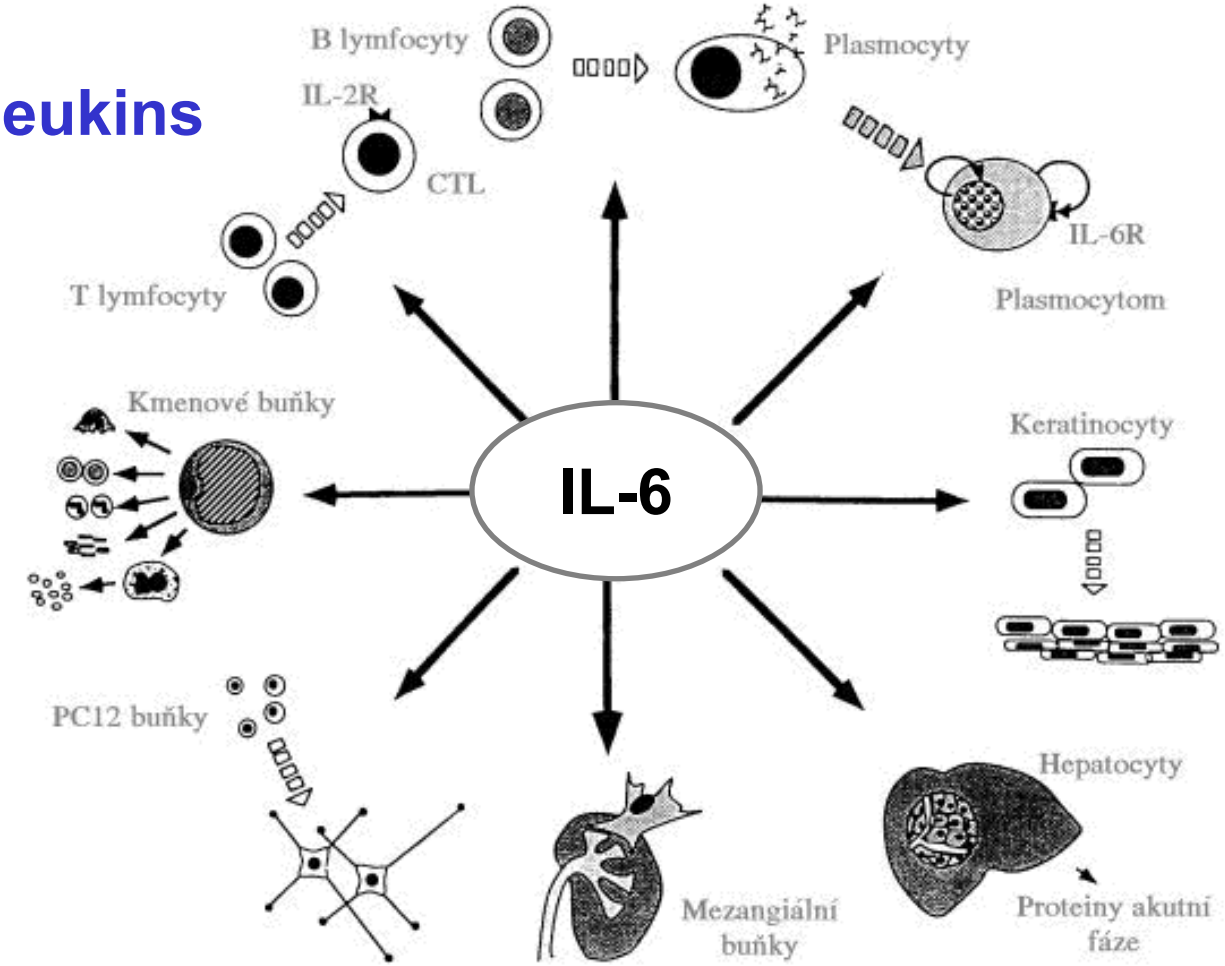
Cytokines

Interleukins



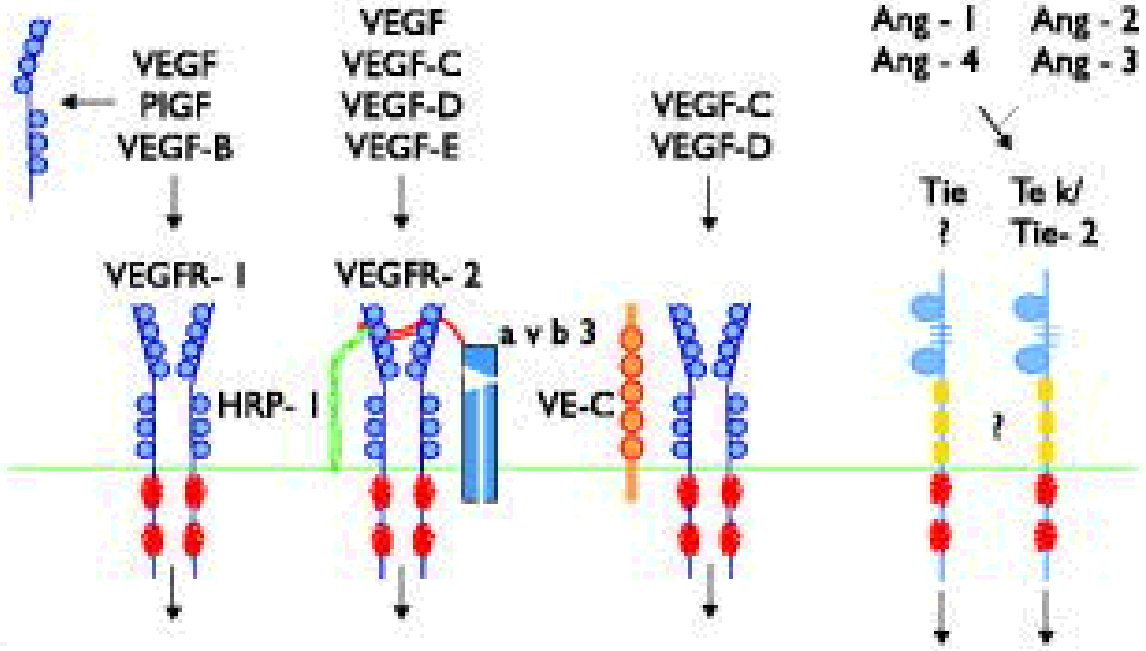
Cytokines

Interleukins

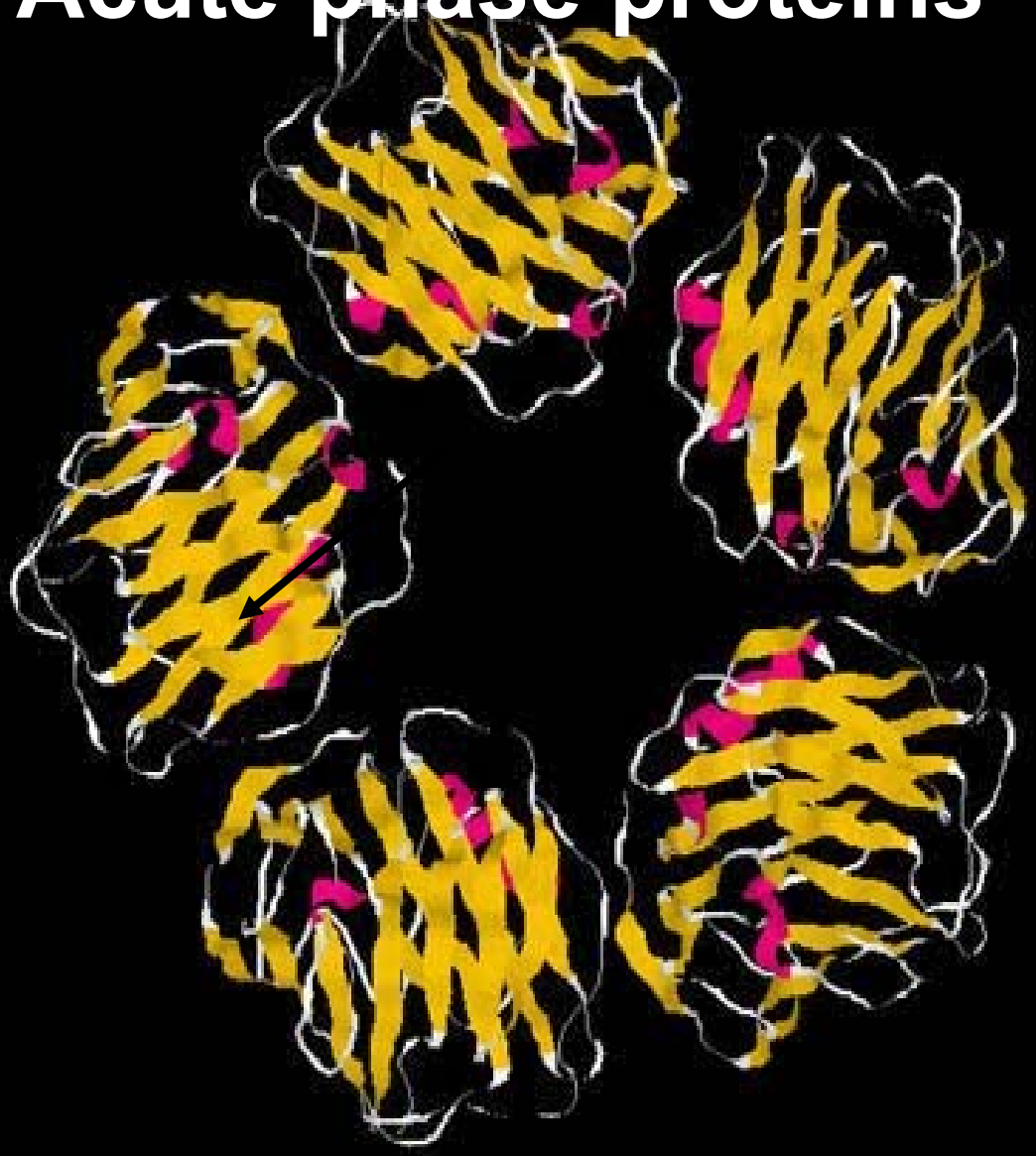


Cytokines

Mesenchymal growth factors



Acute phase proteins



Acute phase proteins

Stimulatory effects of proinflamm. cytokines + corticoids (+ insulin) on hepatic proteosynthesis

An increase of expression of a group of important defense proteins + concurrent depression of both structural and transport protein synthesis

Acute phase proteins = plasma proteins formed in liver; its synthesis is regulated both by proinflamm. cytokines and corticoids

Due to dynamics of changes:

- positive APP (elevation of synthesis)

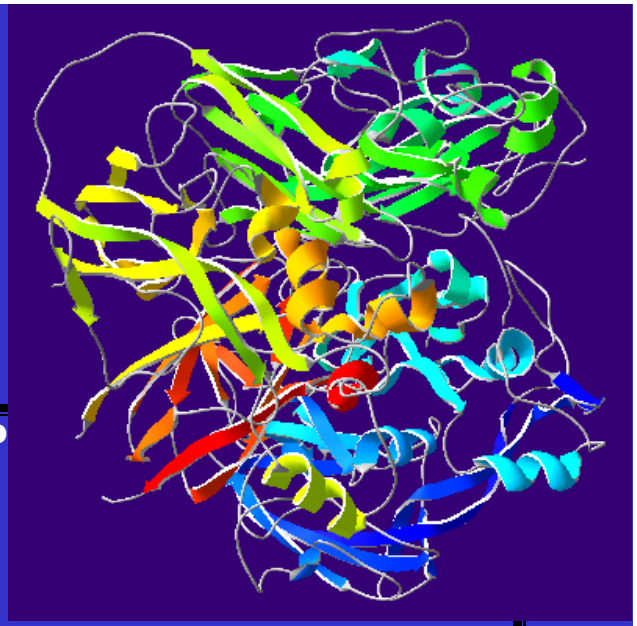
- negative APP (depression of synthesis).

Acute phase proteins

Importance

1. **mediators and modulators** of inflamm. response - members of cytokine cascade (CRP, …)
2. **inhibitors of leukocyte proteases** – limitation of a range of proteolytic tissue destruction (a_1-antitrypsin, a_2-macroglobulin)
3. **scavengers** – binding of both circulating or tissue fragments of damaged cells, hemoglobin fragments (haptoglobin, hemopexin) or free oxygen radicals (ceruloplasmin)
4. some **coagulation factors** (e.g. fibrinogen)
5. **reparatory proteins** – a stimulation of connective tissue proliferation (a_1-acid glycoprotein) and angiogenesis (ceruloplasmin)
6. **transport proteins** - (x CpI); a moiety of other transport proteins (albumin, transferrin) represent negative APP ... their plasma concentration decline during inflammation.

Acute phase proteins



pentraxiny	C reaktivní protein, sérový amyloid P
SERPIN	α 1-antitrypsin (AAT) α 1-antichymotrypsin (AACT) inhibitor proteinu C (PCI) inhibitor aktivátoru plazminogenu 1 α 2-antiplazmin (AP)
metaloproteázy	ceruloplazmin (Cpl) haptoglobin (Hp) hemopexin (Hpx) superoxiddismutáza (SOD)
imunomod. proteiny	α 1-kyselý glykoprotein (AGP) α 2-makroglobulin (AM)
koagulační faktory	fibrinogen (Fbg) von Willebrandův faktor (vWf)
komplement	C3, C4, inhibitor C1 esterázy, faktor B …, vaz.prot. manózy

Acute phase proteins

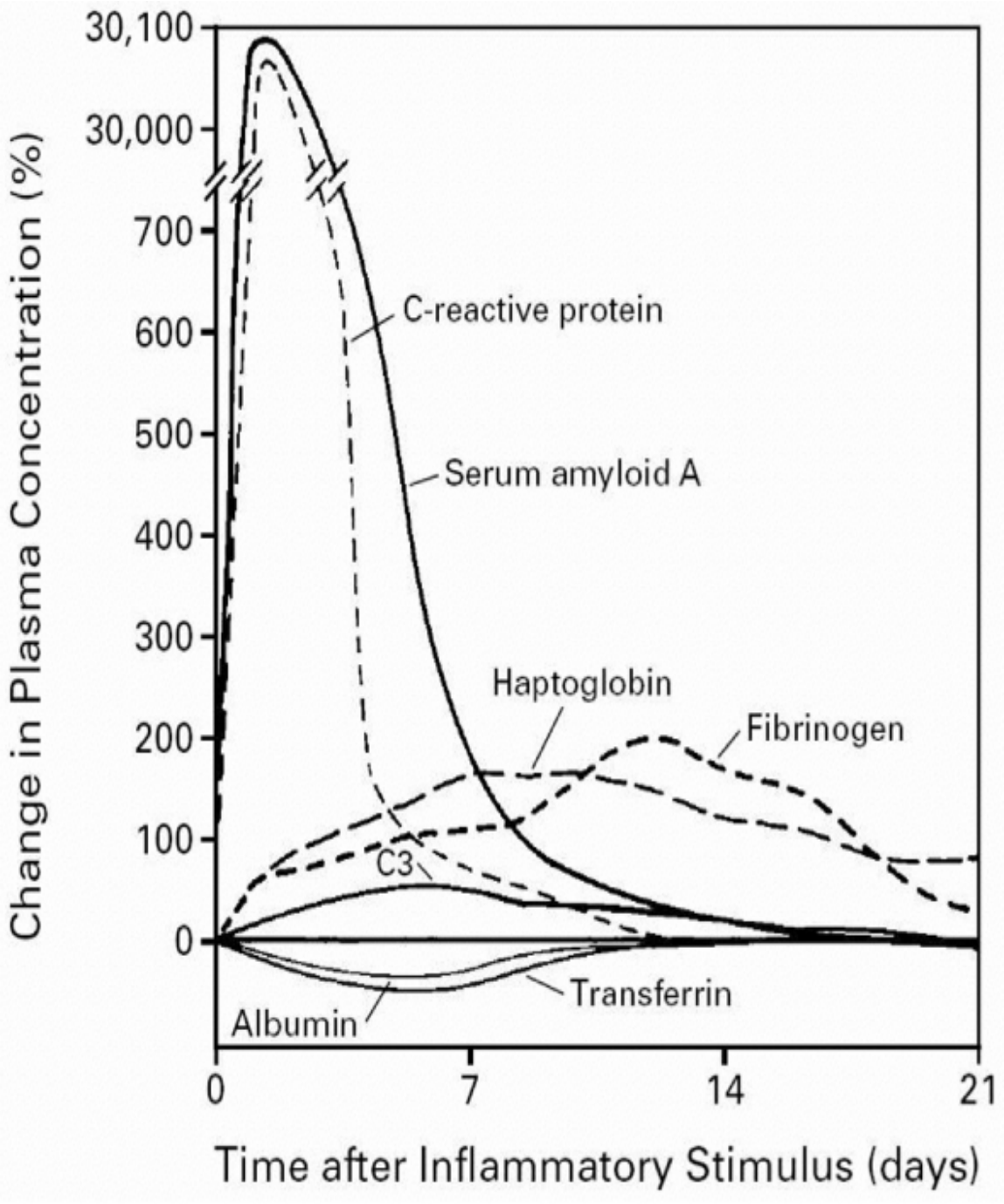
protein	elektrofor.	M_r	konc. (g/l)	poločas[1] (dny)
C reaktivní protein	γ-globulin	110	0,00-0,01	<0,05
sérový amyloid P	γ-globulin	125	0,03-0,04	1
sérový amyloid A		11	0,00-0,02	0,04
ceruloplazmin	α_2-globulin	135	0,12 - 0,28	4 - 10
haptoglobin	α_2-globulin	100	0,3 - 3,0	2 - 4
hemopexin	β_1-globulin	57	1,0 - 1,9	1,8 - 2,5
α_1-kys.glykoprotein	α_1-globulin	44	0,33 - 1,09	5,2
α_2-makroglobulin	α_2-globulin	725	1,2 - 2,4	7 - 8
α_1-antitrypsin	α_1-globulin	52	1,2 - 2,4	3,9
α_1-antichymotrypsin	α_1-globulin	68	0,18-0,26	0,05
PAI-1	α_1-globulin	50	11-69 µg/l	6,7
fibrinogen	β_1-globulin	340	2-4	5,1
albumin	albumin	67	35 - 50	19 - 20
prealbumin	prealbumin	61	0,19 - 0,39	1,9 - 2,7
transferin	β_1-globulin	80	2,2 - 3,6	7 - 9

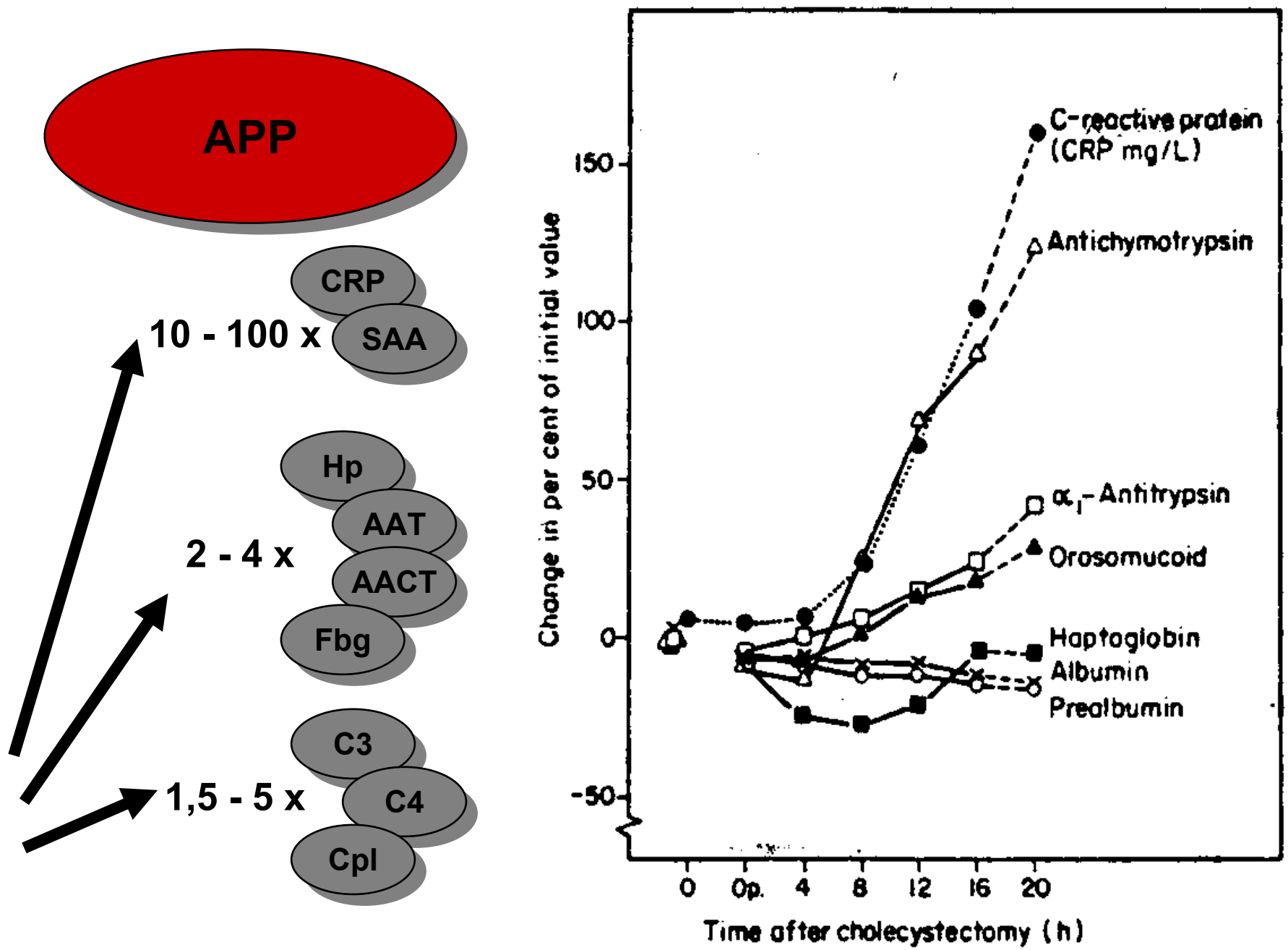
APP

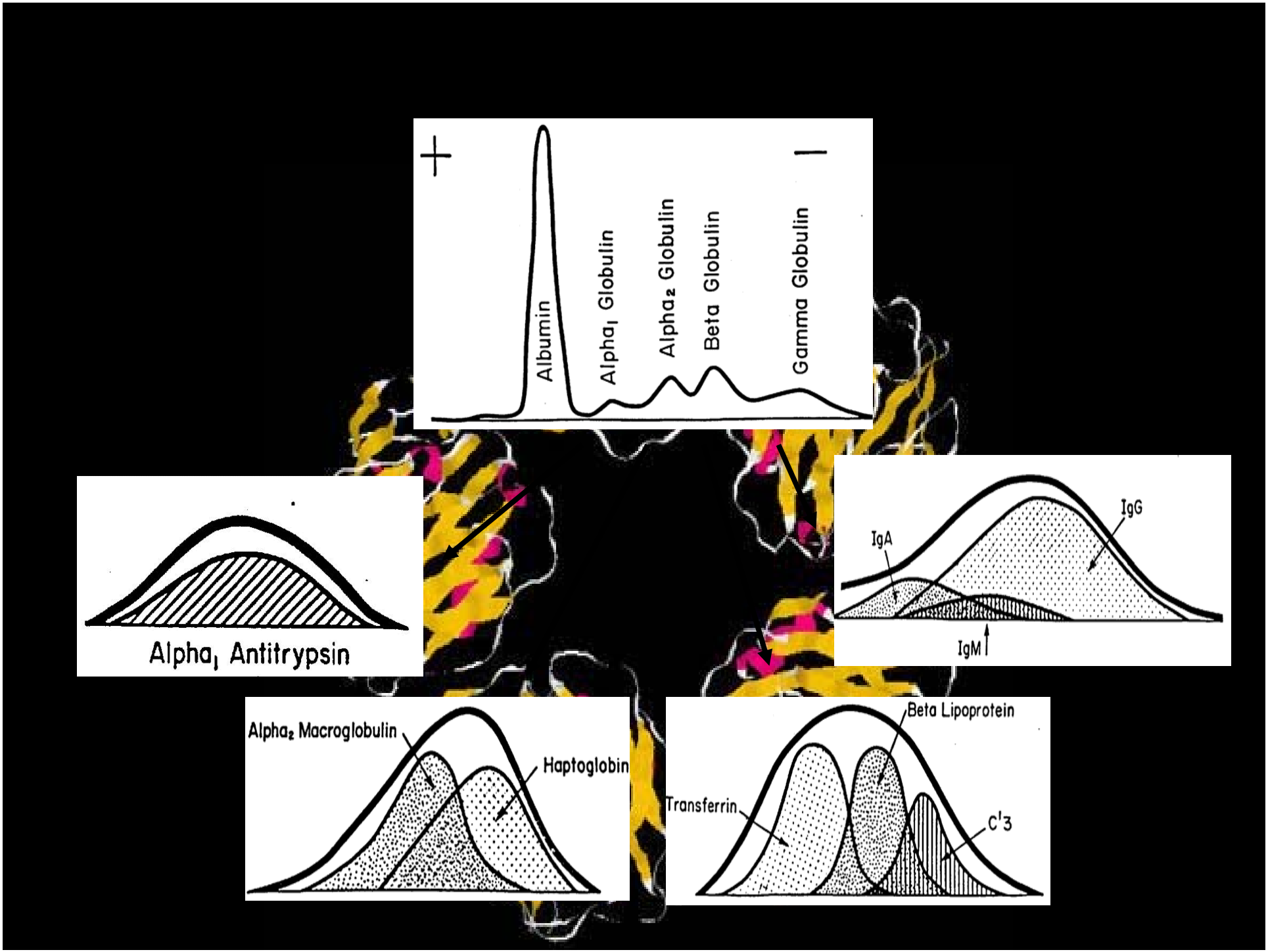
positive

neutral

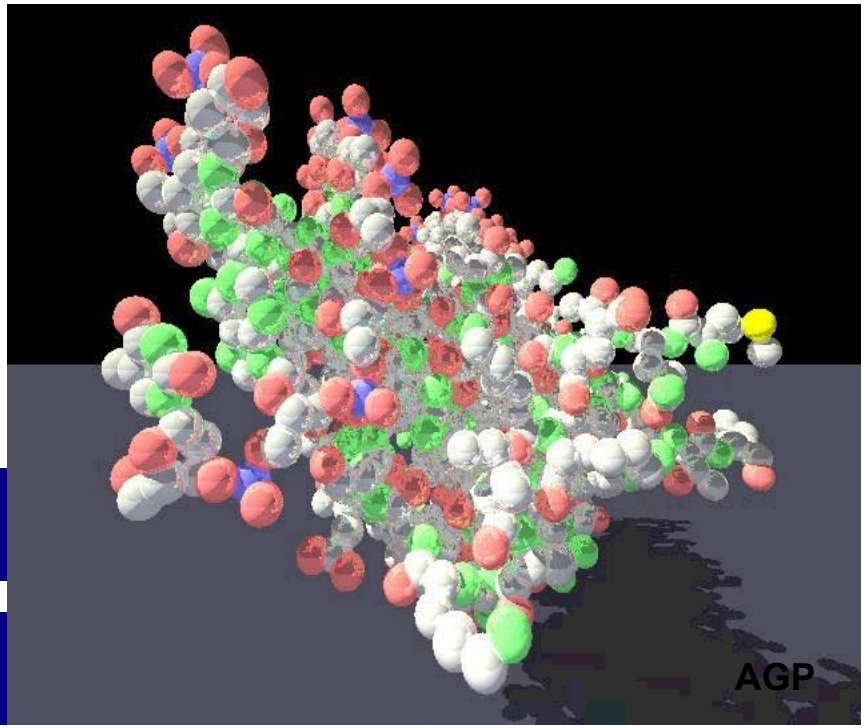
negative




APP
CRP
SAA
10 - 100 x
Hp
AAT
AACT
Fbg
2 - 4 x
C3
C4
Cpl
1,5 - 5 x
C-reactive protein (CRP mg/L)
Antichymotrypsin
α1-Antitrypsin
Orosomucoid
Haptoglobin
Albumin
Prealbumin
Change in per cent of initial value
150
100
50
0
-50
0
Op.
4
8
12
16
20
Time after cholecystectomy (h)

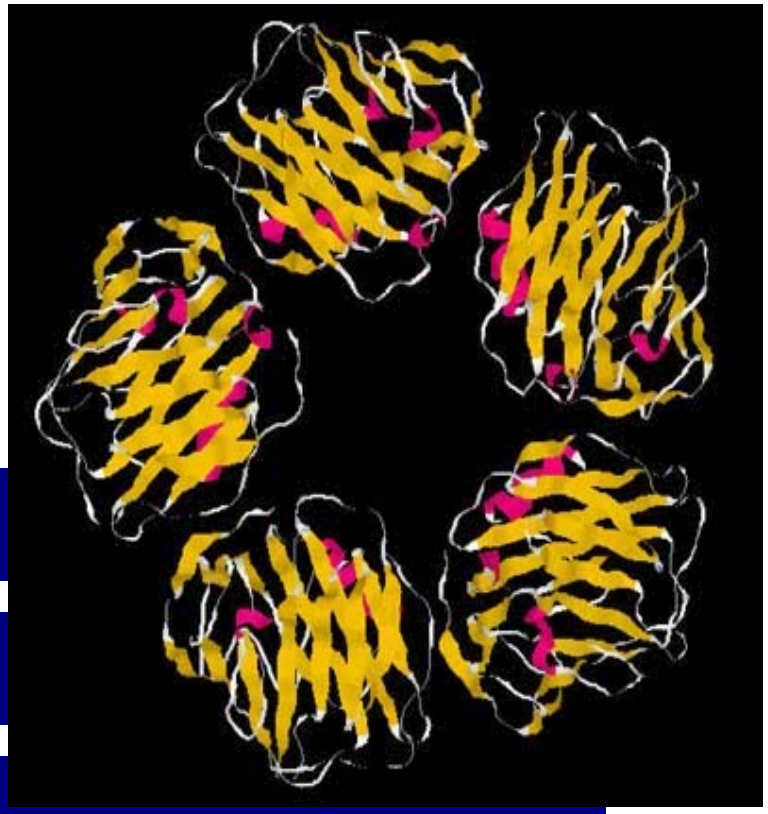

+
−
Albumin
Alpha1 Globulin
Alpha2 Globulin
Beta Globulin
Gamma Globulin
Alpha1 Antitrypsin
IgA
IgG
IgM
Alpha2 Macroglobulin
Haptoglobin
Beta Lipoprotein
Transferrin
C'3

APP



1. pentraxins
2. SERPIN
3. metaloproteases
4. clothing f.
5. complement

APP



1. pentraxins

2. SERPIN

3. metalop

4. clothing

5. complement

CRP
(*Tilet a Frances 1930*)

Serum amyloid P
Long pentraxins

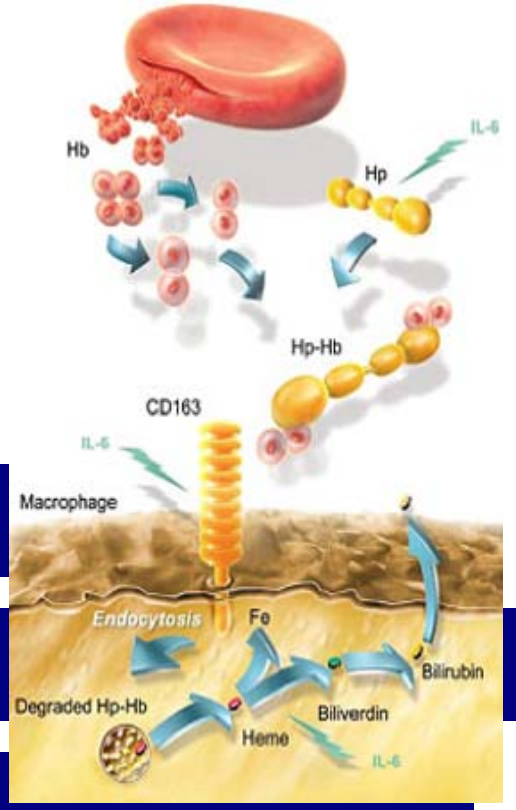
APP

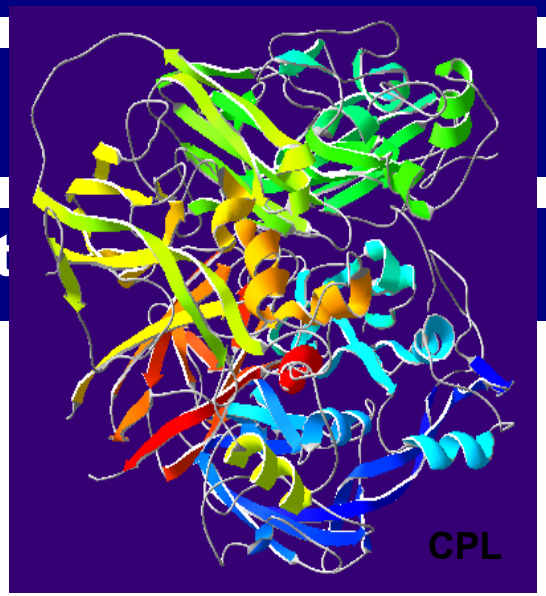
1. pentraxins
2. SERPIN
3. metaloproteas
4. clothing f.
5. complement

- Alpha1-antitrypsin
- Alpha1-antichymotrypsin
- Alpha2-antiplasmin
- PAI-1

APP

1. pentraxins
2. SERPIN
3. metaloproteases
4. clothing f.
5. complement



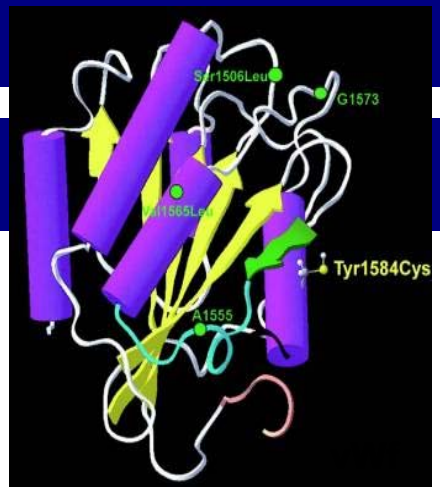


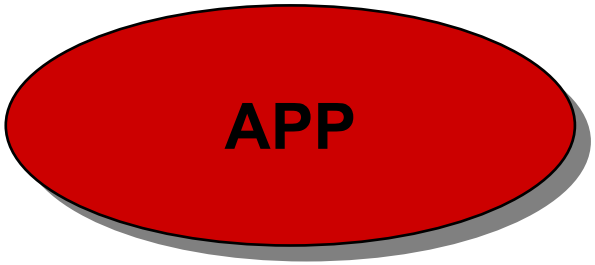
Coeruloplasmin
Haptoglobin
Hemopexin

APP

1. pentraxins
2. SERPIN
3. metaloproteases
4. clothing f.
5. complement

Fibrinogen
von Willebrand f.

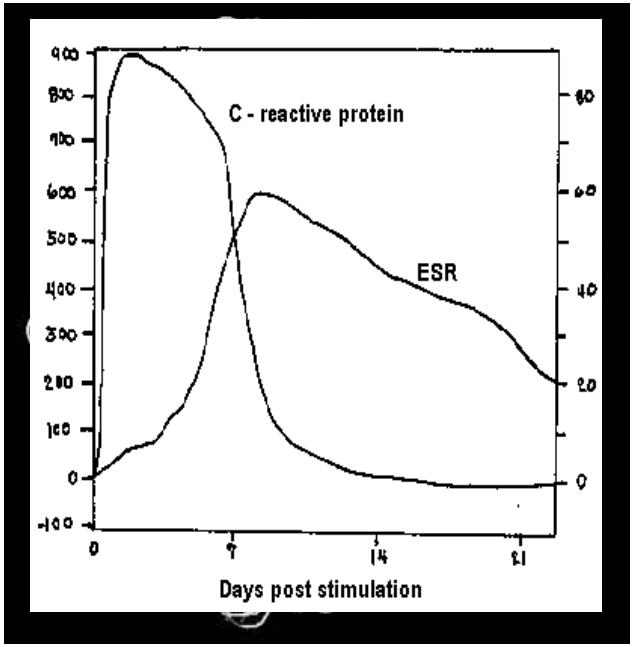




CRP measurement

1. Monitoring of a course and intensity of SIRS
   (infection, inflammation, necrosis)
2. Early detection of (latent) infections in risk patients
3. Diff. diagnostics of bacterial / non-bacterial forms of SIRS
4. Screening of risk patients (e.g., before surgery)
5. Risk factor of cardiovascular diseases

APP



CRP in diagnostics

- Increase 4-6 h after inflam. stimulus
- Normalisation within 3-7 days
- Upto 100-times increase from basal levels
- Direct quantification of acute phase response
- Measurement possible in serum and plasma

- Low specificity to inflamm. stimulus
- Physiological range is stabile independent to age
- No influence of anemia, polyglobulia proteinemia

Sedimentation rate

- Slow response
- Normalization in weeks
- Indirect measurement of acute phase reaction on the basis of Fbg changes
- Full blood is needed for „on line“ measurement
- Low specificity to inflamm. stimulus
- Gender and age differences (female, elderly pt.)

Fever

Old defence reaction, well conserved in evolution

Correlation with better prognosis and duration of acute infection

Defense reaction → adverse environment for microbes, for their metabolism and proliferation.

Fever

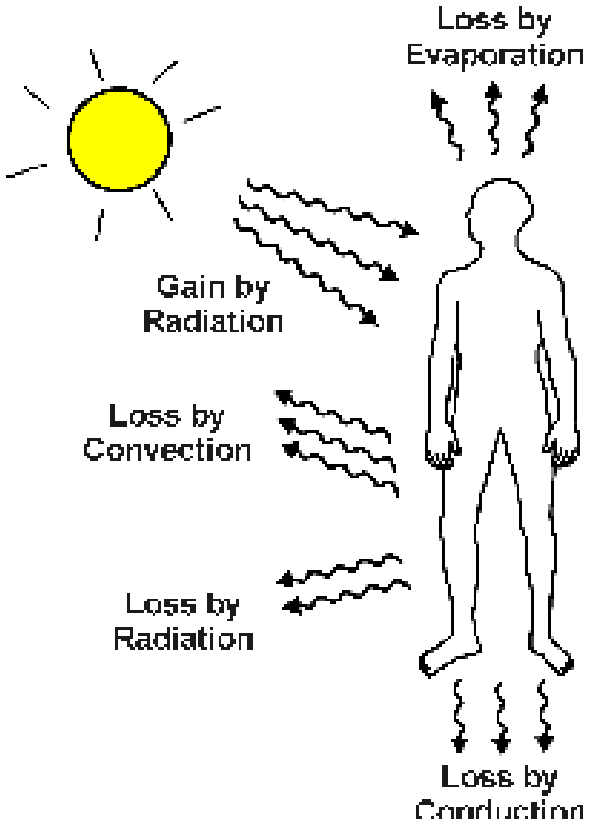
Regulation of temperature

heat production

(intensity of metabolism – catecholamines, $T_{3,4}$)

x

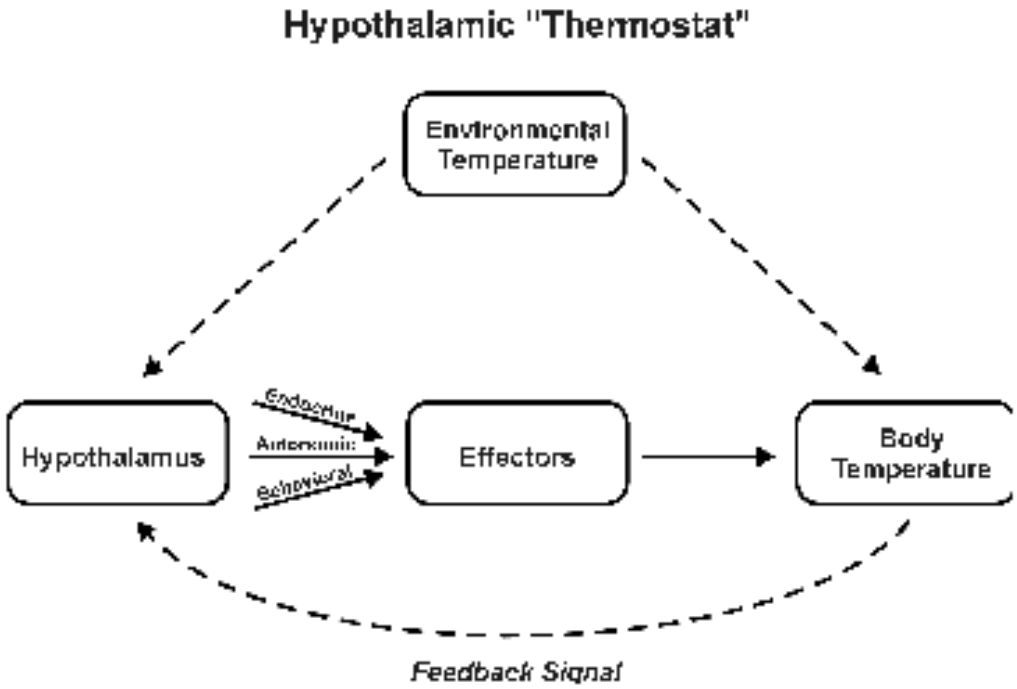
heat output



Fever

Hypothalamic thermoregulatory center

- „thermostat“



Fever

TNF, IL-1, IL-6 + microbial toxins + PG

↓

stimulation / reset of hypothalamic center

•direct effect of mediators in hypothalamus

•indirect / afferent stimulation via n. vagus from periphery to hypothalamus

↓

• peripheral vasoconstriction (cold skin, hypoperfusion)

• heat production (thermogenesis)

↓

Restoration of initial status

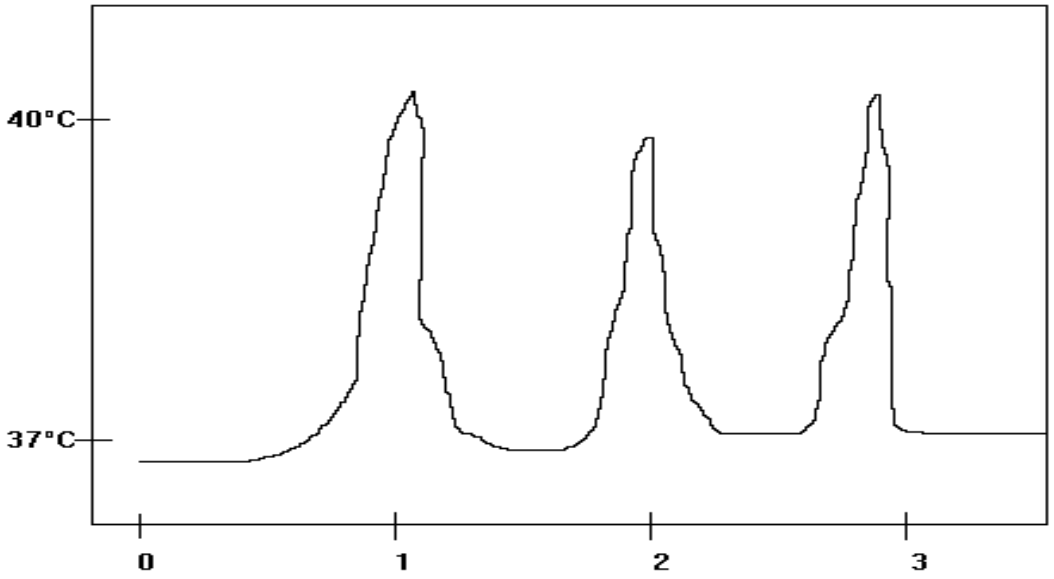
•vasodilatation

•perspiration

Fever

Main types

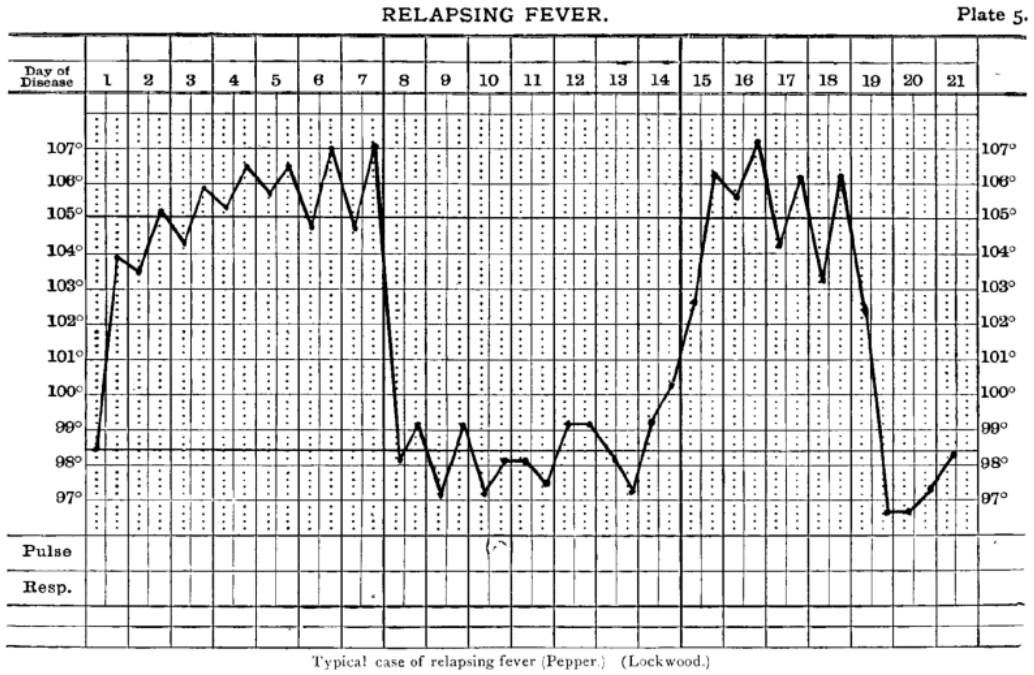
- **febris remittens - daily differences > 1°C**



Fever

Main types

- **febris intermittens - fever periods ←→ normal temperature**



Fever

Main types

- **febris continua - fluctuation < 1°C**