Imnunology 3

Innate immunity mechanisms

Introduction

- Barriéres
- **<u>Nonspecific mechanisms</u>**

- activated or almost activated
- starting at once
- recognition of broad spectrum of agenses
- destruction or phagoctosis and destruction
- inflamation (localisation of infection)

Cellular mechanisms

- Innate immunity engages cells mediated mechanisms
- Receptors recognising ligands (antigenic molecules) of pathogens start inflamation and destruction of microbes by **fagocytes**.
- **NK bunky** – kills infected and transformed cells.

Recognition of antigens

- **PRR - Pattern recognition receptors** – present on cells of nonspecific immunity
- **PAMP – pathogen associated molecular patterns** – molecules present on foreign cells
- Innate mechanisms are able to recognise self and nonself

PAMP

- Peptidogycan
- LPS
- non specific antigens of viruses and other microbes

PRR

Extracelular proteins on membranes

bound on phagocyting cells **receptors**

- **TLR** – recognition of broad spectrum of pathogens., transmission of signal to the nucleus, activation of genes encoding production of cytokínes stimulating inflamation
- **opsonins** – molecules binding on the surface of microbes, that make them more attractive for phagocytes

Several receptors can be activated at once

Preformed receptors

- present as part of **innate immunity**
- enable rapid reaction

PRR – on soluble molecules and host cells

TLR – present on host cells

KAR – on NK cells

KIR – on NK cells

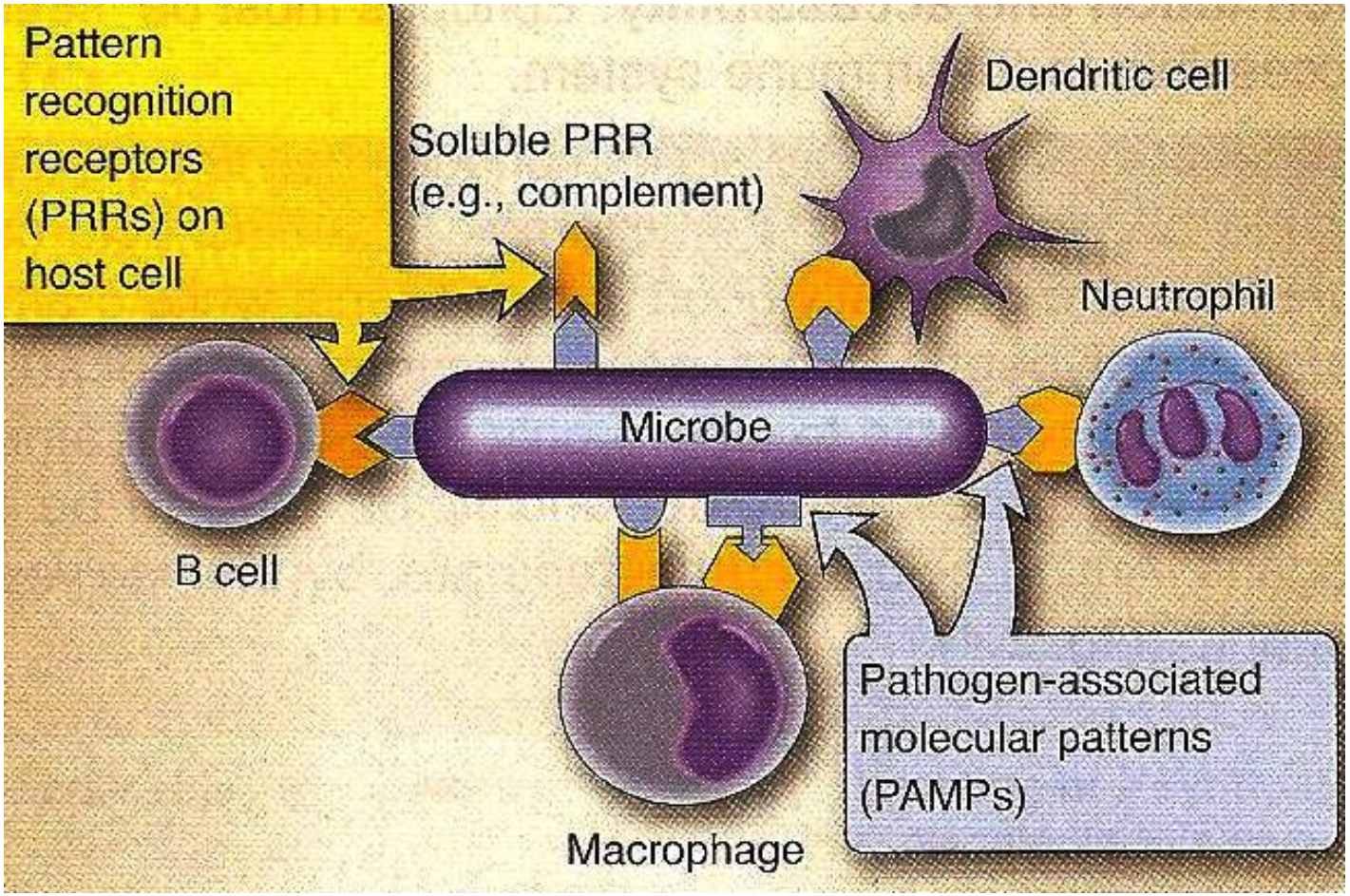
CR – on soluble molecules, phagocytes, on B cells

FcR – on phagocyting cells

PRR – pattern recognition receptors

- present on host cells or in soluble forme (proteins of complement
- recognise different motives (patterns) present on microbes and not on self cells PAMP – pathogen associated molecular patterns
- this bound starts different forms of inflamation with the aime to kill the pathogen

PRR – pattern recognition receptors



TLR – toll like receptors

- present on host cells
- bind PAMP of microbes
- start transcription, synthesis and secretion of cytokins stimulating inflamatin and attraction of macrophages, NK cells, neutrofils and dendritic cells to the site of infection

Molecules detecting changes of self

- molecules of thermal shock, molecules MICA, MICB – produced:

- by cells infected by virus
- cells on cancerogenesis

Recognised by TLR of phagocyting cells

activating KAR - Killer Activating Receptors on NK cells

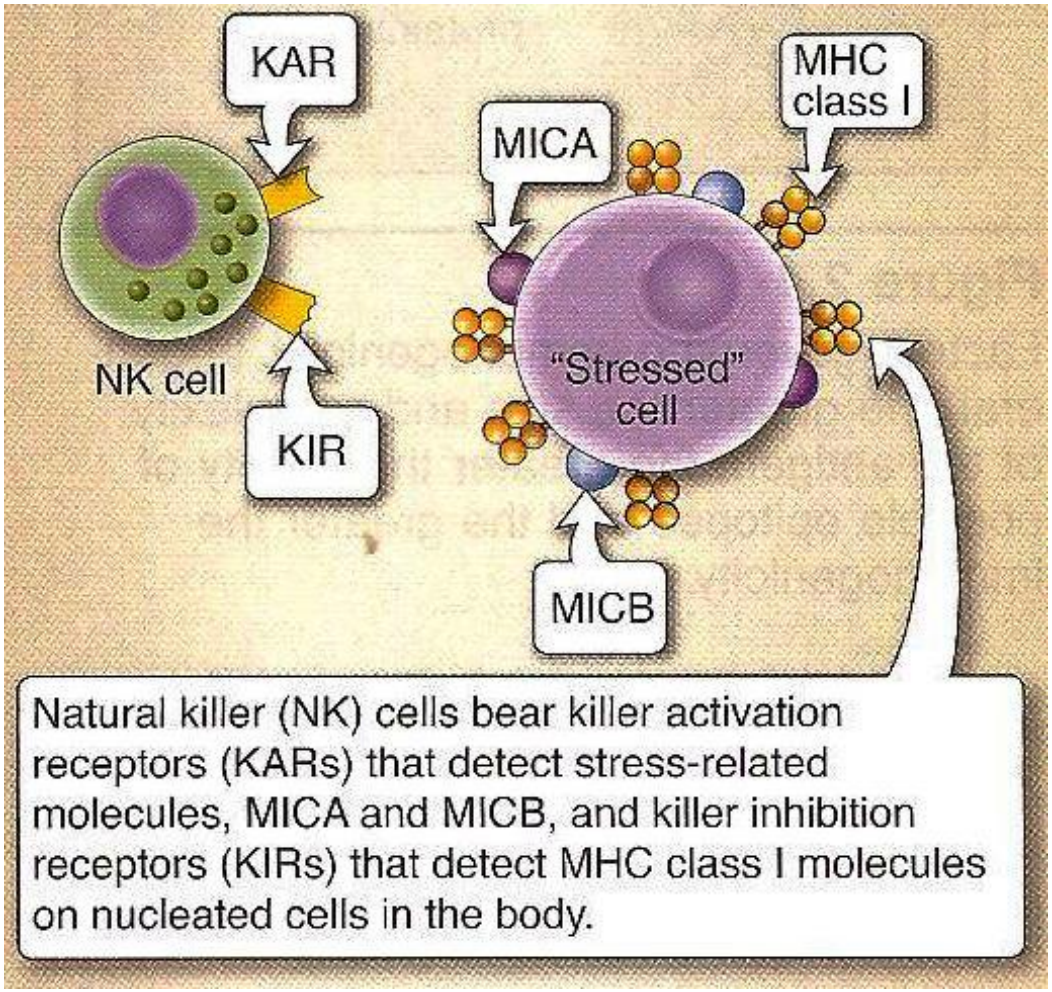
NK cells

- NK cells recognise changed cells and direct them to destruction
- NK cells **contain KAR – killer activation receptors** – that recognise stress molecules, incl. **MICA a MICB** in on surface of inected and transformed cell

KIR, KAR on NK- natural killers

- NK cells – part of lymphocytic line
- Receptors recognising changes on self cells molecules (after viral infection, or changed by Ca - gennic processes)
- **KAR** – **killer activation receptors** – recognise MICA, MICB (stress molecules) on self cells that activates NK cells to kill self cell.
- **KIR** – **killer inhibition receptors** – monitors MHC I molecules. Tumor and virus infected cells decrease number of MHC I, that decrease the possibility of binding to MHC I molecules and decrease the inhibítion of killers

KIR, KAR – on natural killers NK



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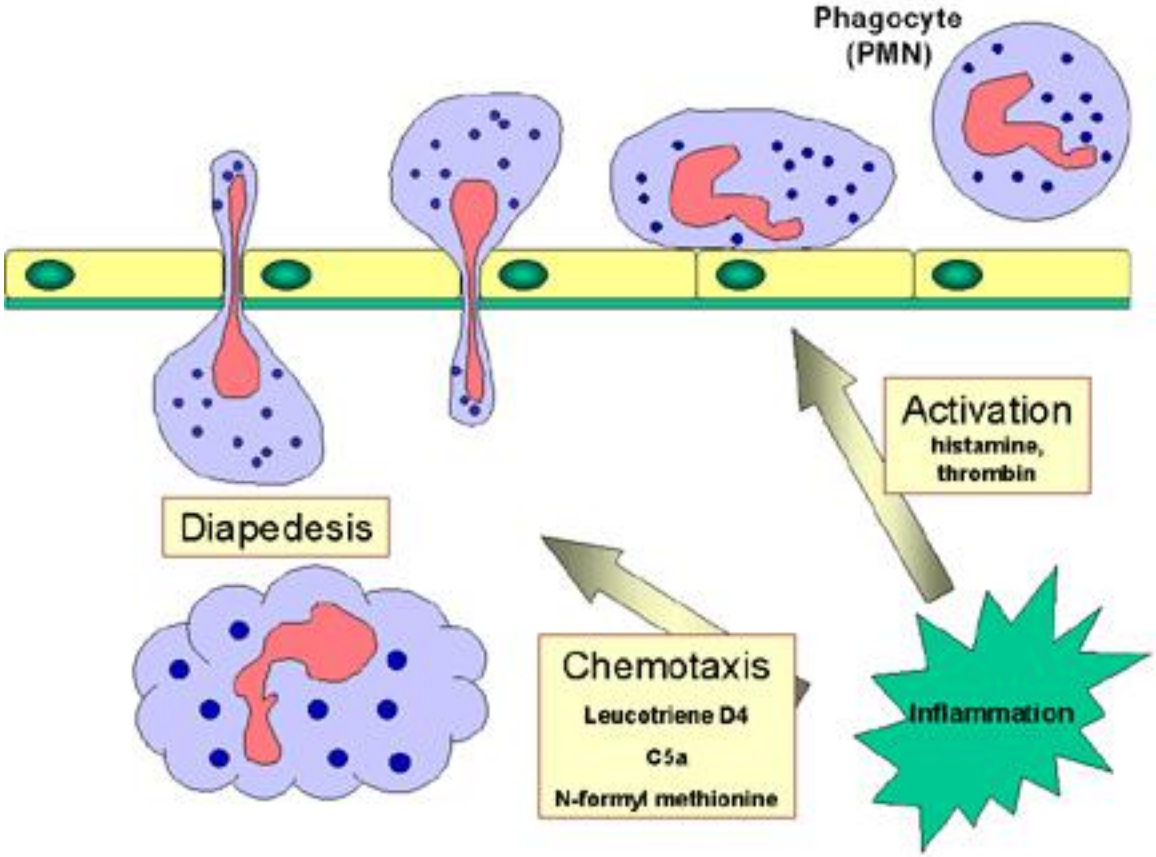
Fagocytosis

- engulfment and degradation of microbes and other corpuscular parts of cells: macrophages, dendritic cells, neutrofils and even B lymfocytes

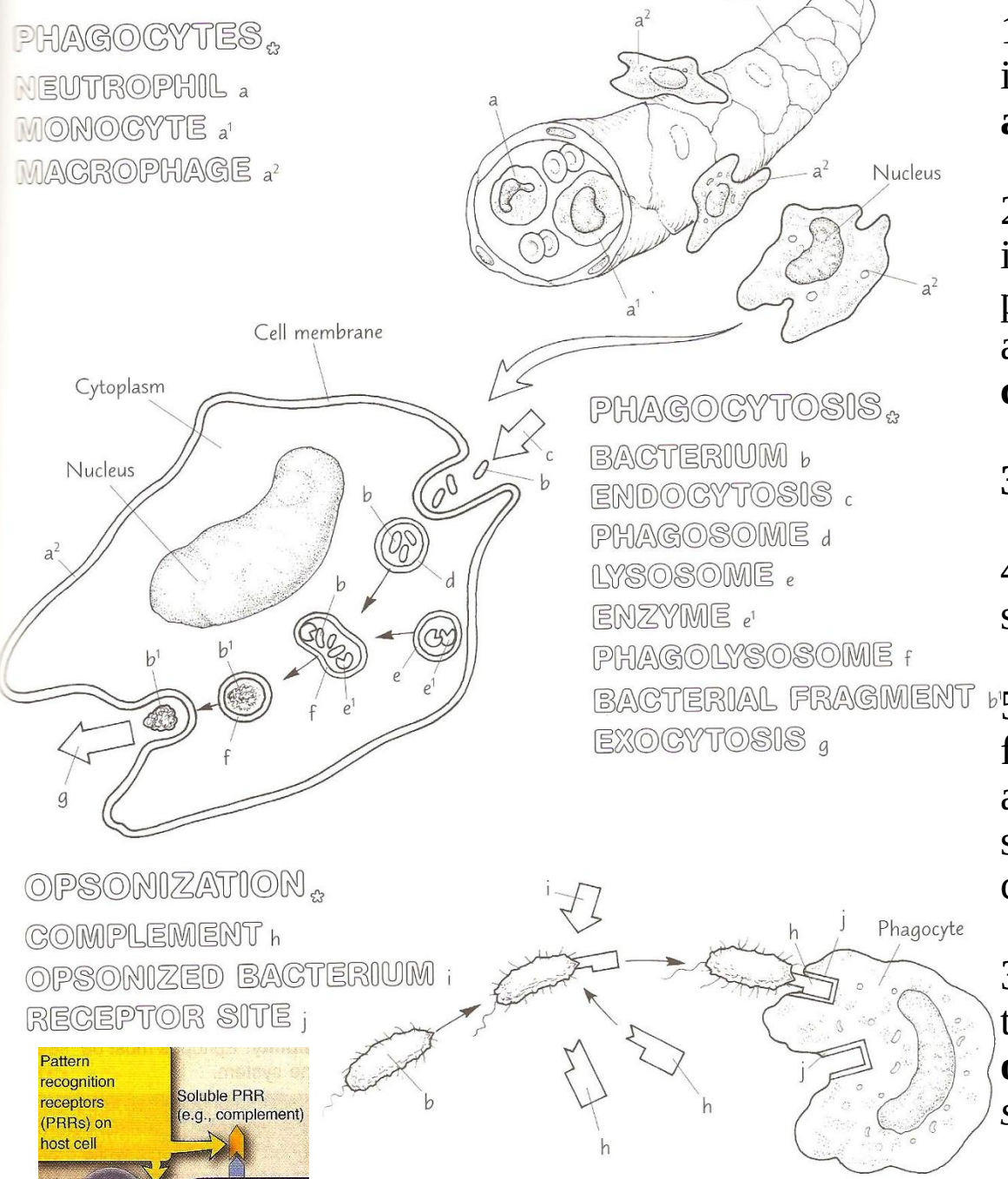
Are part of cleaning mechanisms of the body

- protect body by ingestion
- disrupt rests of cells and parts

- 1. **translocation** of leu throught intercellular binding of endotel and **adherence** on endotel
- 2. enter leu in interstitium and its activation by chemical substances produced in sites where microbes are present as answer to disruption - **chemotaxis**
- 3. antigen is covered by molecules that make ingestion easier - **opsoniation** *fibronectin, **IgG specific antibodies*, C3
- 4. adherence of Ag on neutrofils starts - **internalisation,**
- 5. formation of **fagosomes** and their fusion with primary (hydrolytical and proteolytical enzymes) and secondary granules (lactoferin, cytochrome b)


Phagocyte
(PMN)
Activation
histamine,
thrombin
Diapedesis
Chemotaxis
Leucotriene D4
C5a
N-formyl methionine
Inflammation

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3

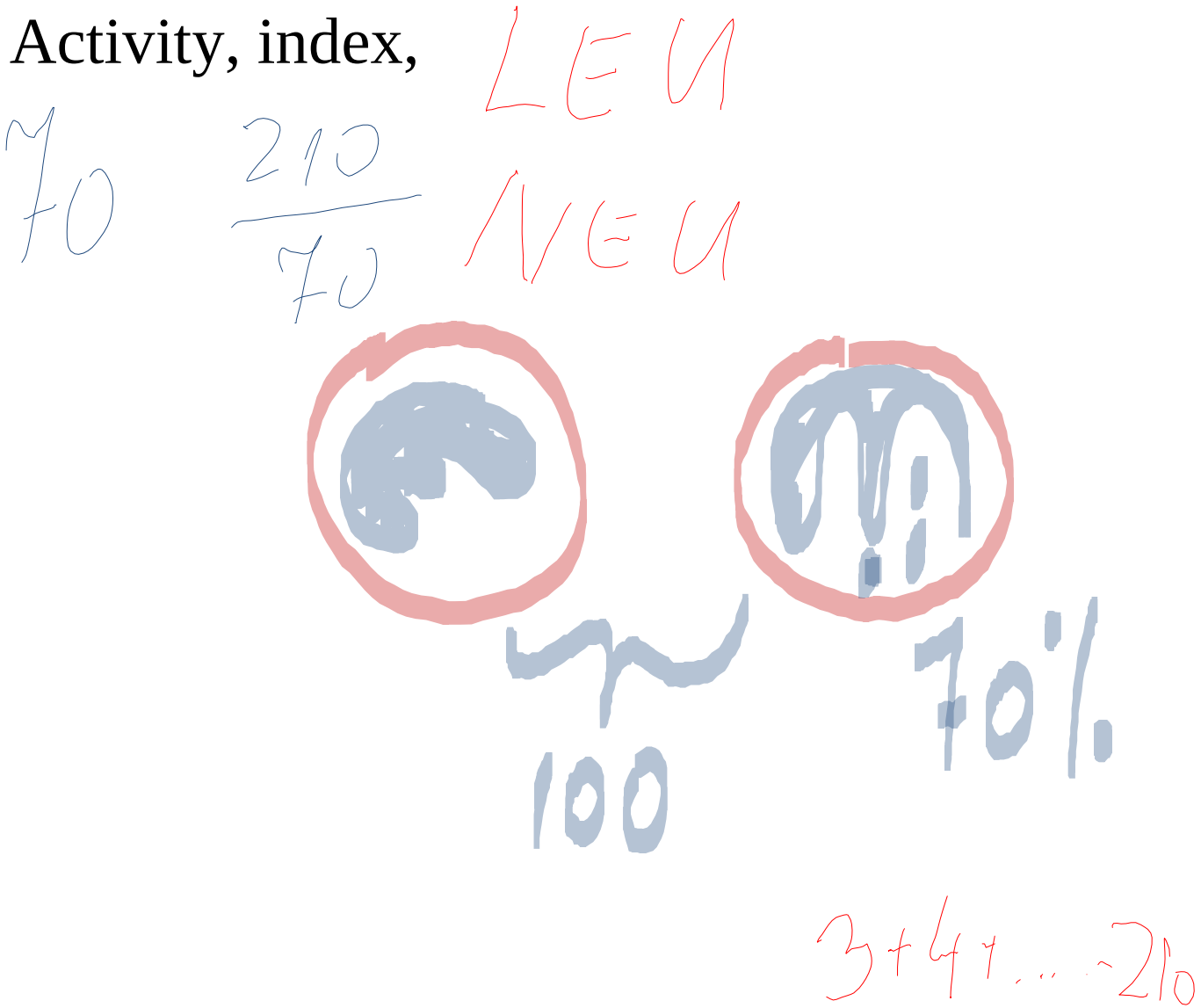
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Demonstration

- Phagocytosis – Activity, index,



Fagocytosis, activity and index.

- Leukocytes (PMNL) are offered the foreign bodies
  - in a way that full blood is incubated with baker yeast.

- The foreign bodies are phagocyted by PMNL.

- The blood smear is done and stained by May Grunwald method.

- Under microscope with imersion we read the picture.

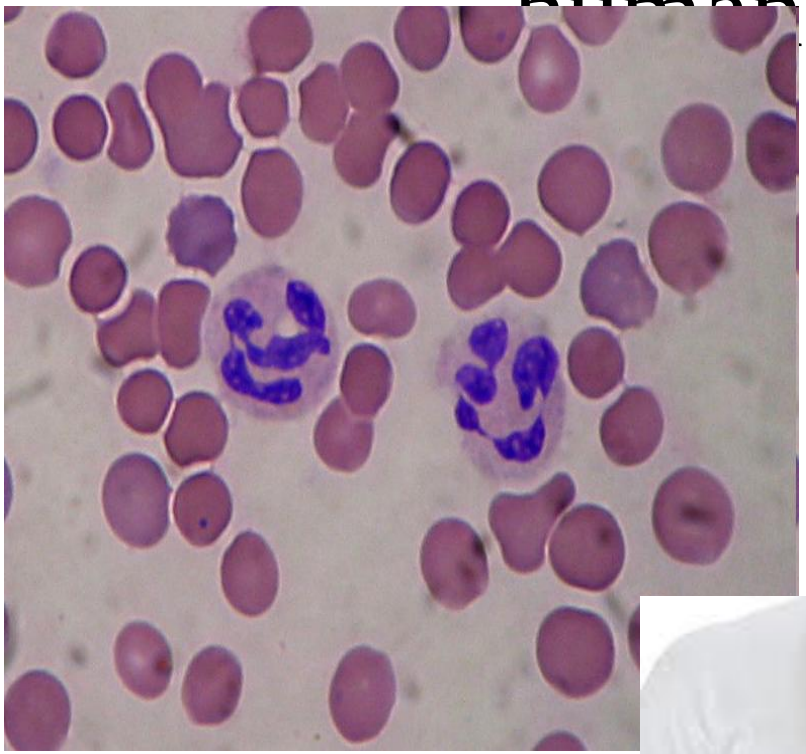
Fagocytosis, activity and index.

- No of phagocyting PMNL and not phagocyting PMNL are counted untill 100. No of phagocyting PMNL in % is determining the phagocytosis or phagocytic activity.

- In phagocying PMNL we register by adding the no of foreign bodies – yeast that are phagocyted – localised intracellularly.

- The fagocytosis index is calculated by dividing the no of phagocyted foreign bodies by no of phagocyting PMNL (%).
- This is the value of functional properties of phagocytes.
- Normal FI=2,5

Phagocytosis of candida albicans by human nuetrophils

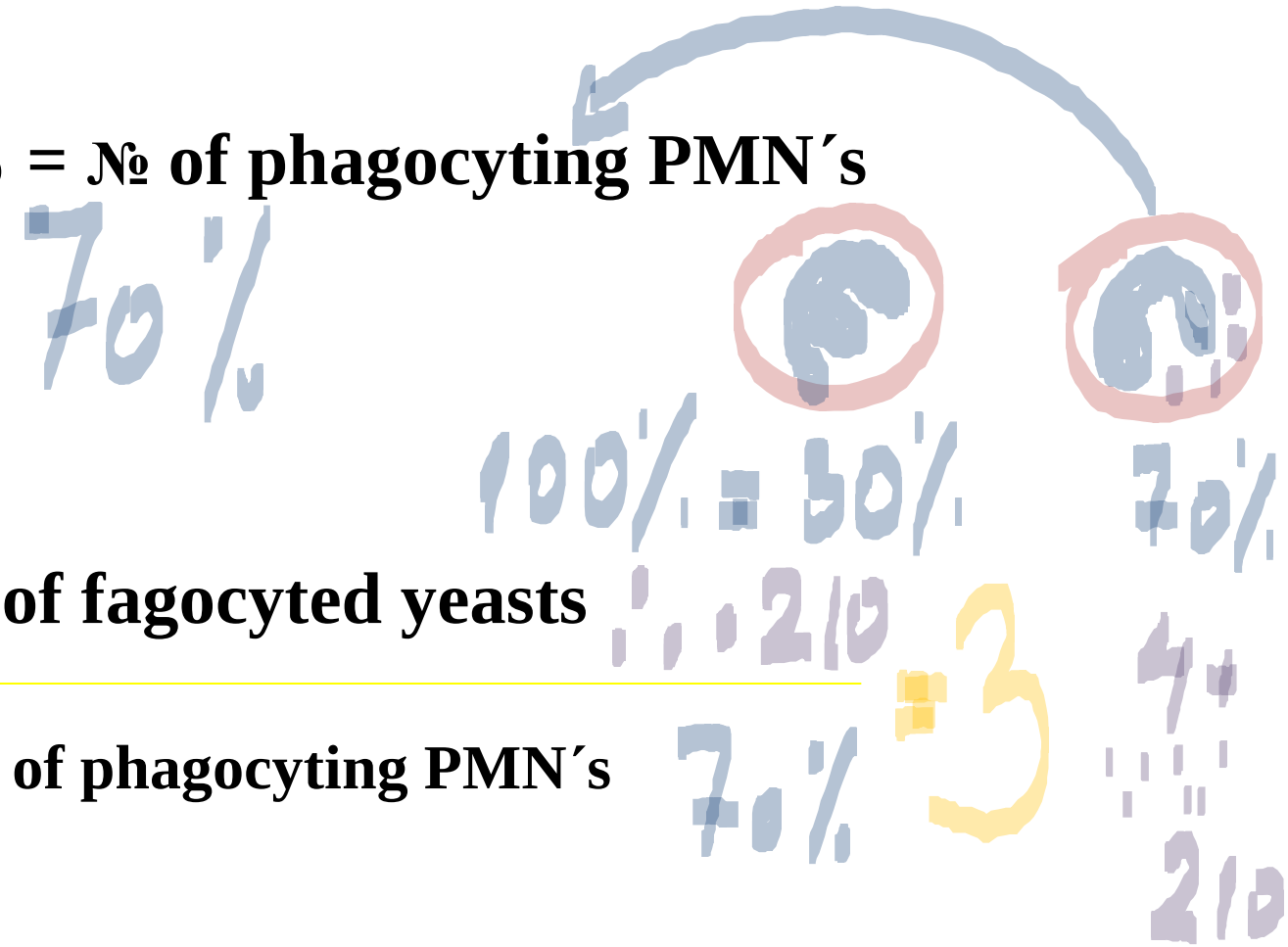






Phagocytosis – activity, index

FA % = № of phagocyting PMN´s



$$FI = \frac{\text{№ of fagocyted yeasts}}{\text{№ of phagocyting PMN´s}}$$

FA = 77,1 +- 8,8 %

FI = 3,6 +- 0,8

Fagocytosis, activity and index.

- Normal level of FI =2,5
-
- If less – after longlasting bacterial infections (no sources)
- - in-borne deficiency (lazy neutrofils)
- If more – activated in ongoing acute infection

Humoral mechanisms

- Besides activity of cells, innate machanisms engage also soluble molecules

- destruction of cells (infected by virus)
- increase activity of other mechanisme (Phagocytosis)

Interferon type I

Defensins

Cytokines

Complement

Interferon type I

- produced by subgroups of dendritic cells (IFα), fibroblastes (IFγ)
- after binding viral PAMP and PRR
- start activation of antiviral mechanisms (activity of macrofages, dendritic cells...)

Defensins

Many cells: **epitelial, neutrofils, macrophages in skin or mucose membranes** secrete peptids rich on cysteín

These peptides form chanals in cell membrane of bacteria this enable influx of ions and bacterial death.

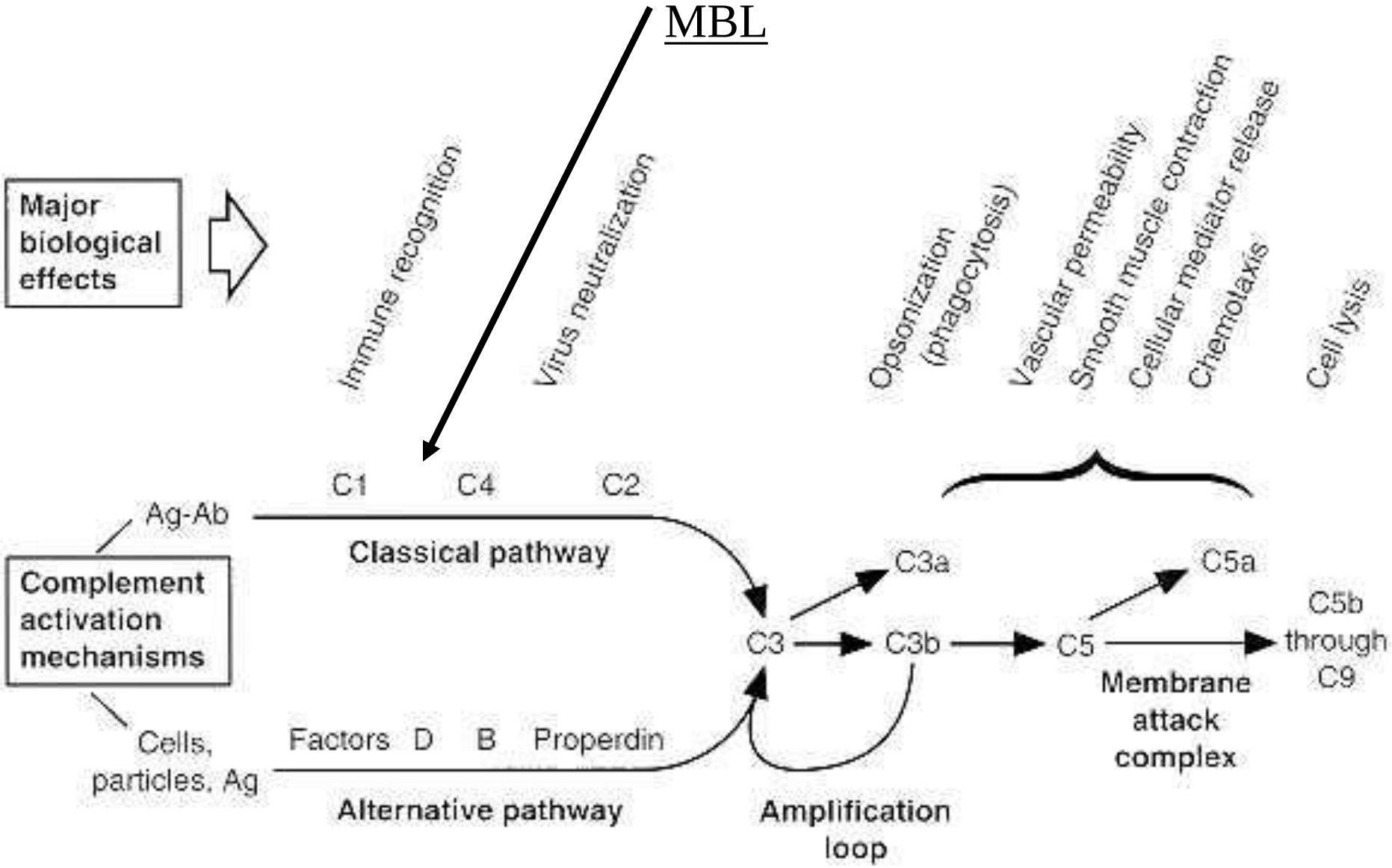
Other molecules with microbicidal functions: **lysosym, Dnase, Rnase**

Cytokines and chemokines

- **Cytokines -** secreted by leucocytes and other cells
  - engaged in innate immunity, adaptive immunity a inflamation
  - antigen nonspecific way
  - induction of broad spectrum of physiological changes.
- **Chemokines** subgroup of cytokines with low molecules
  - engaged in chemotaxis (chemically induced migration)

Complement

- System of enzymes and proteins engaged in specific and adaptive immunity mechanisms
- In innate mechanisms this systeme is activated by
  - alternative path
  - MBL (manan binding lectin) path


MBL
Major biological effects
Immune recognition
Virus neutralization
Opsonization (phagocytosis)
Vascular permeability
Smooth muscle contraction
Cellular mediator release
Chemotaxis
Cell lysis
C1
C4
C2
Ag-Ab
Classical pathway
Complement activation mechanisms
C3a
C5a
C3
C3b
C5
C5b through C9
Membrane attack complex
Cells, particles, Ag
Factors D B Properdin
Alternative pathway
Amplification loop

Complement

- Consists of innactive circulating glycoproteins activated in cascade way after innitial stimulation

- 3 paths of stimulation:
  - c*lassic (antigen + antibody*)
  - alternative (microbes and their products)
  - MBL path

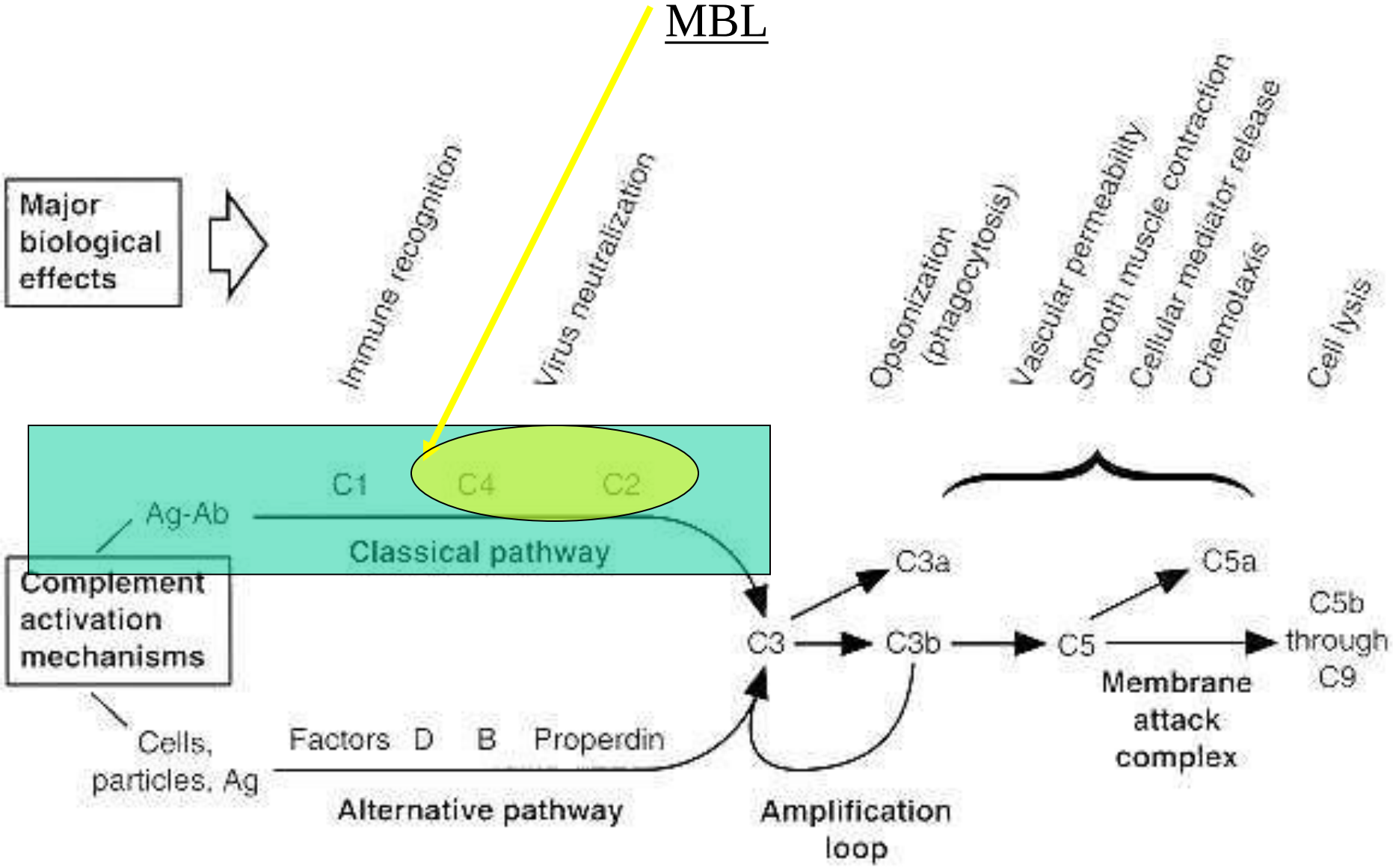
- Results in production of:
  MAC membrane attact complex
  it is bound on cell surface (of microbe) formation of disruptions in surface memgrane that leads to lysisof cell

C

- - Complement components C1 to C9, B, D a Properdin are proteins present in serum
- - Fragments of native components of complement are described by small letters,C3b Bb). Smaller fragmentsa “a“, bigger fragments by letter „b“.
- - Horizontal line is indicateing proteins or complexes of complement with enzymatic activity $\overline{C4bC2b}$.

Alternative path

- Initiated by cell surface products recognised as foreign for host cell (LPS)
- Many enzymes (calicreín, plasmin, elastasa) disrupt C3 part of serum complement present concentration 1300 mg/l in several small fragments i.e. shorty living and not stabil C3b fragment.
  It is important **opsonin** binding on receptors on cell surfaces


MBL
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Mannose Binding Lectin – path

Activated by binding of **lectin on** mannose in rests of glycoproteins of some microbes *Listeria*, *Salmonella*, *Candida albicans.*

MB**L** is protein of acute phase inflamation.

MB**L** bound on mannose cooperates with **Mannose Activated Serine Protease** to activate C2, C4 a C3

C´--lectin--mannose (*Listeria*, *Salmonella*, *Candida*)

MAC – end product of C cascade

- C5a — chemotaxis anaphylatoxin
- C3a
- **C3b**C5bC6C7C8C9
- opsonisation

Lysis – end effect of C activity

- Binding of C5b on bacterial membrane starts formation of MAC and lysis
- C5b start consecutive attachement of C6, C7 a C8.
- C8 is responsible for firme anchoring in the membrane and attachment of C9 molecule, then the pores in membrane are formesd.
- Disrupted integrity of membrane results in nonregulated flow of electrolytes and lytical death of cell

Inflamation

- - activation of complement by alternative or lectin binding path,
- - attraction and activation of phagocyting cells that secrete cytokines and chemokines,
- - activation of NK cells,
- - changes of vascular permeability
- - rise of body temperature

Signs

ache

warm

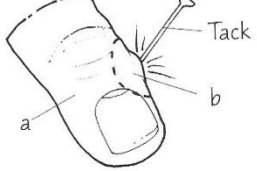
redness

oedema

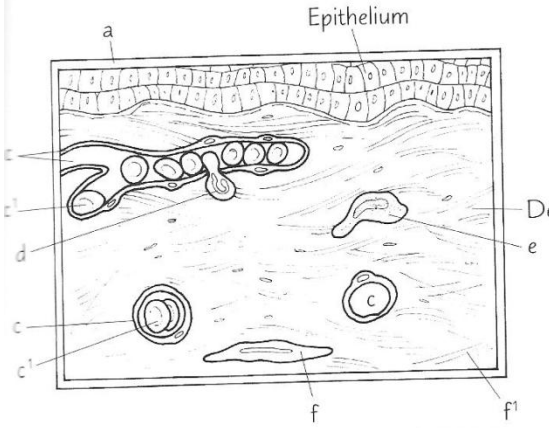
loss of function

(dolor, calor, rubor, tumor, functio laesa)

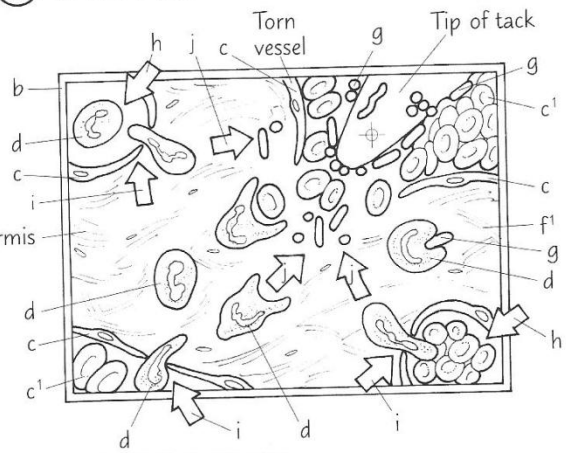
INFLAMMATION ☆



① NORMAL SKIN a

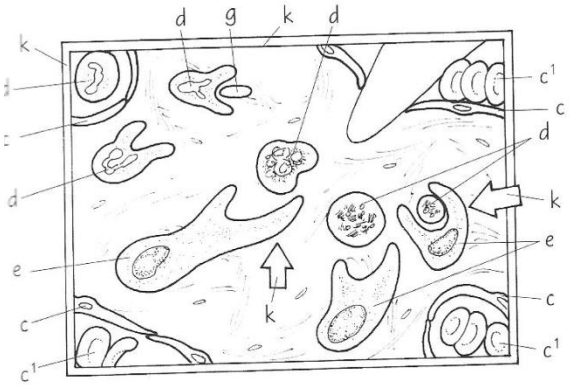


② INFLAMED SKIN b

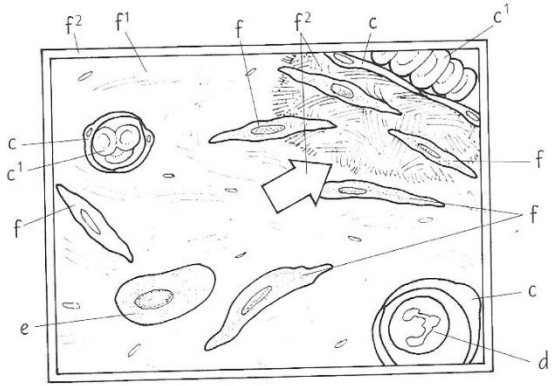


BLOOD VESSEL c / RBC c¹
NEUTROPHIL d
MACROPHAGE e
FIBROBLAST f / FIBER f¹

BACTERIUM g
VASODILATATION h
DIAPEDESIS i
CHEMOTAXIS j



③ PHAGOCYTOSIS OF DEAD NEUTROPHILS k



④ HEALING: FIBROSIS f²