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Cell, Inflammation, Wound healing

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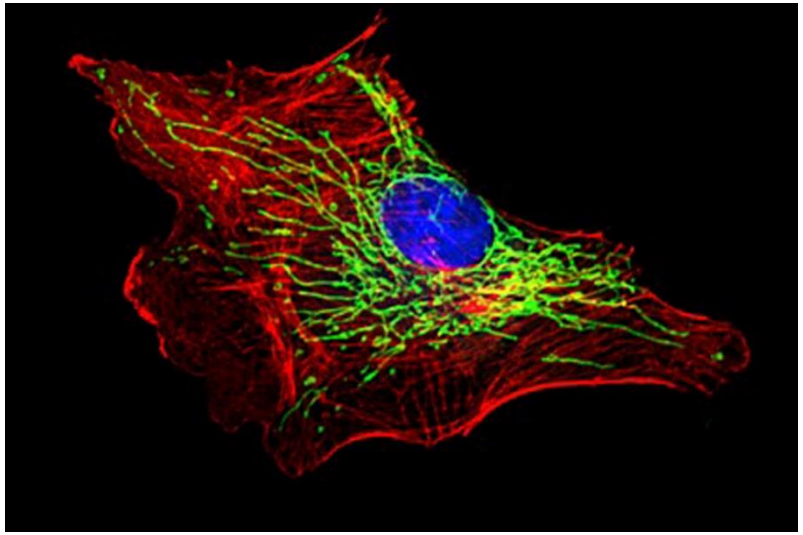
Cell

Mitochondrial function/dysfunction
ROS
Hypoxia
Lysosomal function/dysfunction
Cell death

Mitochondria



Mitochondrial network

- production of ATP for cellular energy needs
- metabolism of amino acids
- regulation of the redox state of cells
- heme synthesis
- differentiation and activation processes of immune cells
- crucial functions in the cell death program

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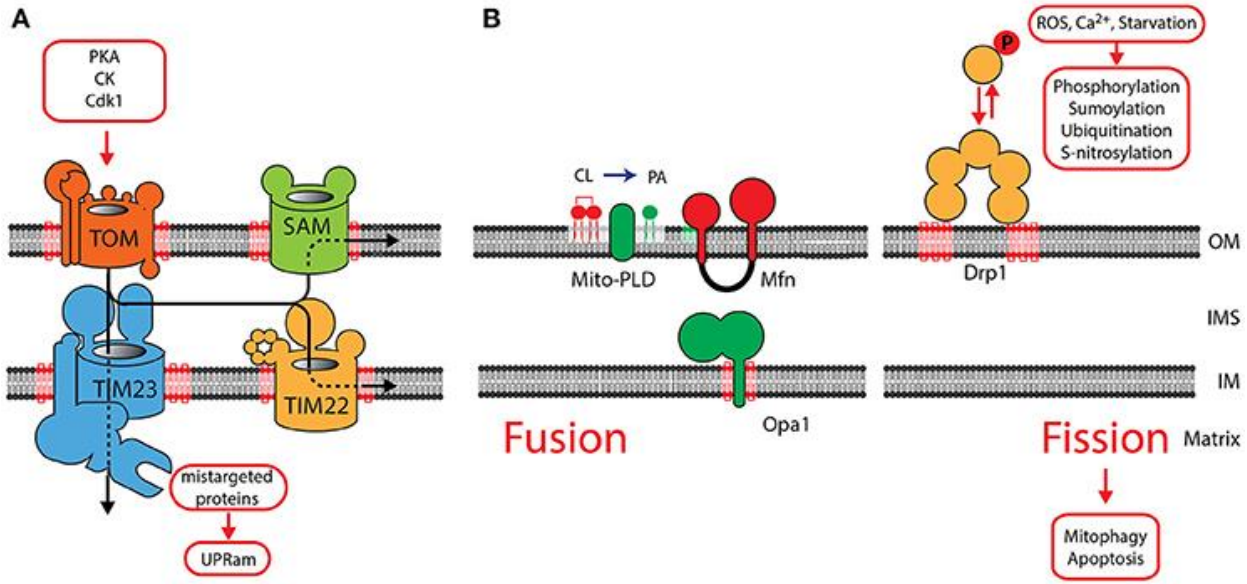
Mitochondrial fusion and fission

- processes occur in response to various extra- or intracellular changes
- changes in nutrient supply, energy or redox status, during cell differentiation in a cell-type dependent manner



Front. Cell Dev. Biol., 2017

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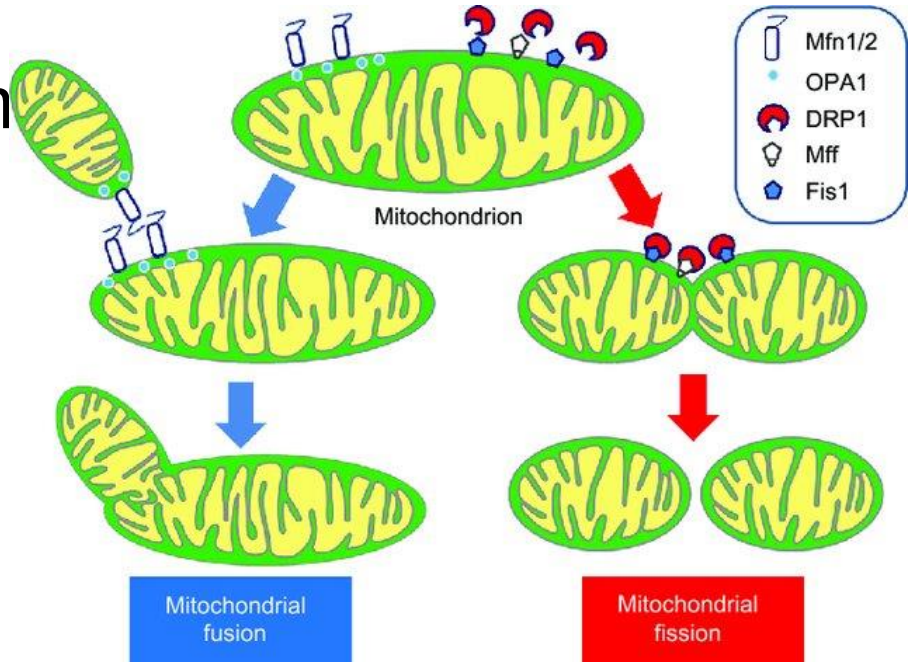
Mitochondrial fusion and fission

- response to metabolic/pathogenic condition
- FUSION = autonomously integrate
- 1. fusion of the outer membrane between 2 adjacent mitochondria
  - mediated by mitofusin 1 and 2
- 2. fusion of the inner membrane
  - cardiolipin, dynamin-like GRPase optic atrophy (OPA)
- important for maintenance of mitochondrial DNA integrity and cellular respiration

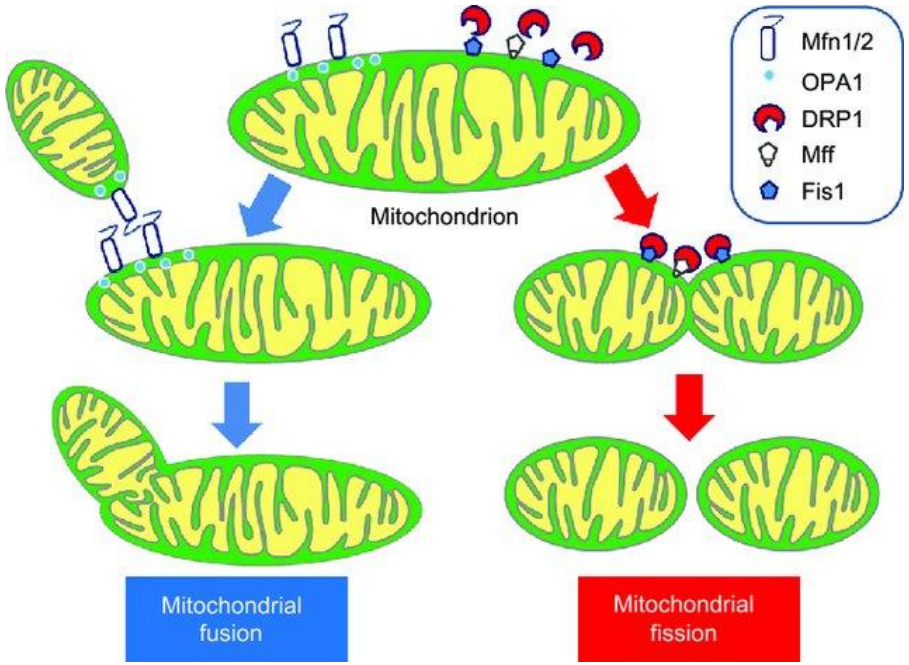


Research Reports in Clinical Cardiology 2014(default):111

MUNI MED

Mitochondrial fusion and fission

- FISSION
- important to allow inheritance of mitochondria by daughter cells during cell division
- when damaged and deleted - damaged mitochondria facilitates their removal by mitophagy



Research Reports in Clinical Cardiology 2014(default):111

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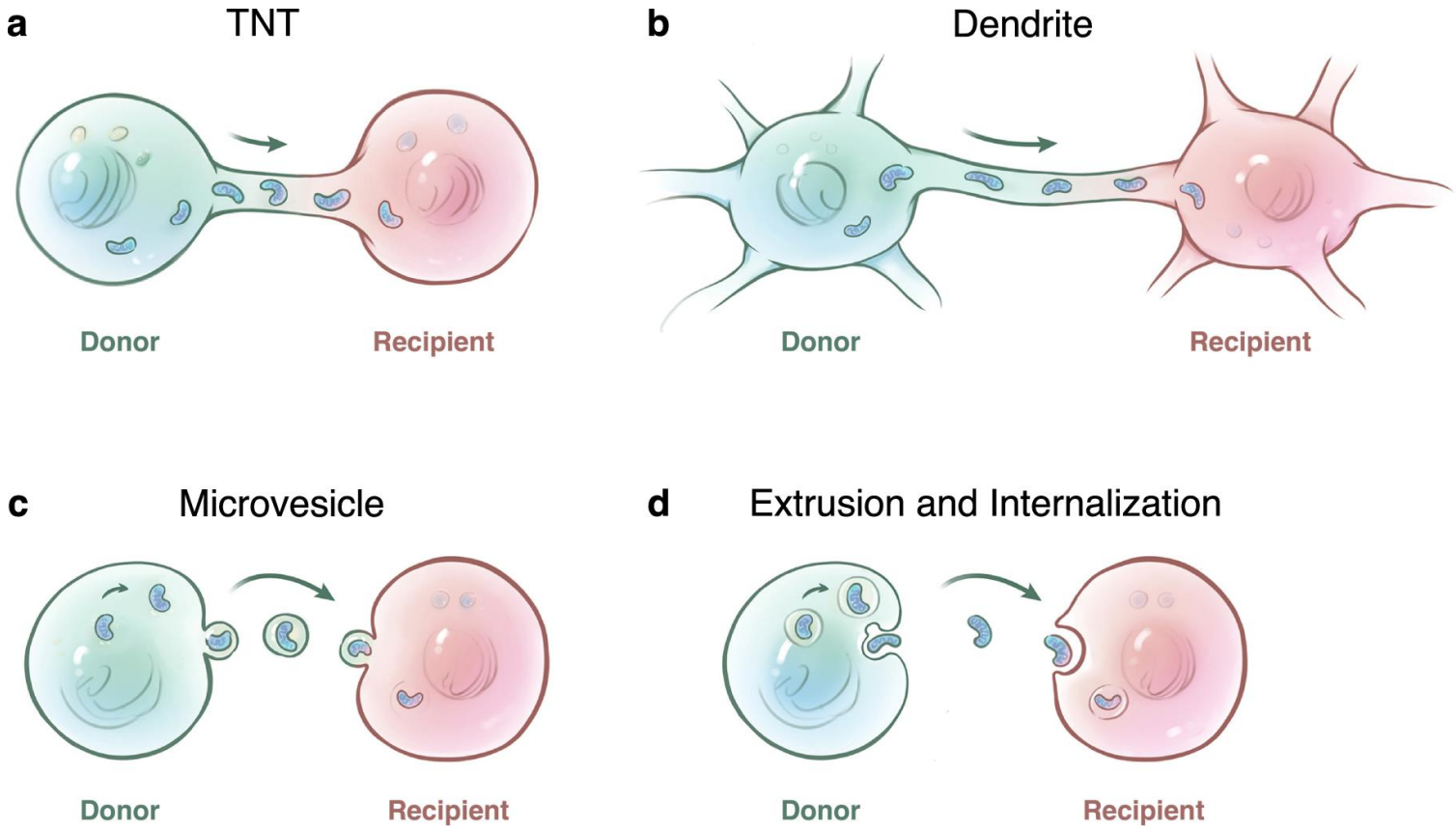
Routes of mitochondrial transfer from donor cells to recipient cells



a TNT is a membranous tubular protrusion that extends from the plasma membrane, with a variety of diameters between 50 and 1500 nm and lengths from several tens to hundreds of microns. TNT is the most popular route of mitochondrial transfer between the connected cells. **b** Dendrite is another form of the membranous protrusion. Some cells with dendrites (e.g., osteocytes) are connected to each other via intrinsic dendrites to form an intercellular network, which provides a highway for mitochondrial transfer. **c** Microvesicles formed by blebbing of the cellular plasma membrane were reported as another route for mitochondrial transfer. **d** Free mitochondria alone can be extruded or internalized without carriers, which provides a possible route for intercellular mitochondrial transfer

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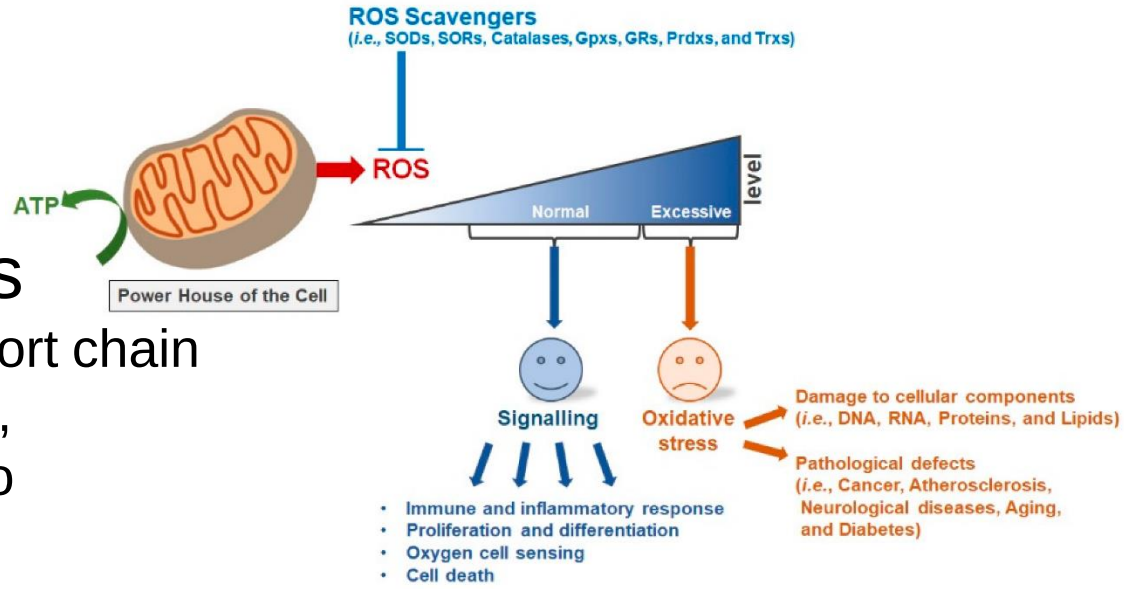
Mitochondria and ROS



Int. J. Mol. Sci. **2019**, *20*(18), 4407

- production of reactive oxygen species
  - generated by mitochondria via the electron transport chain
  - byproduct during mitochondrial energy production, consequence of fatty acid β-oxidation, exposure to radiation, light, metals, and redox drugs
- ROS function:
  - second messengers in various signaling pathways
    in immune cells: Ca^{2+}-NFAT signaling pathway, which is critical in T cell activation.
  - ROS can also damage bacterial pathogens, but
- if produced excessively - damage the producing cell or neighboring cells.

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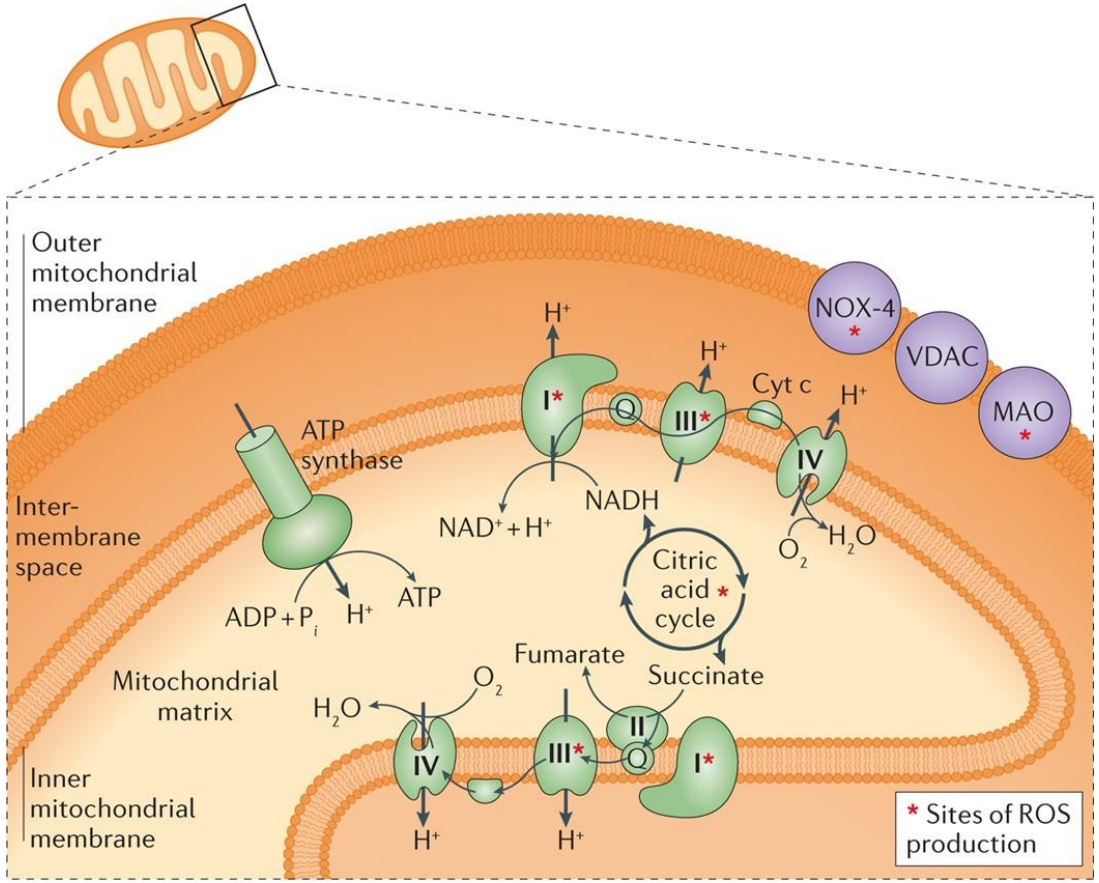
Sites of ROS production

- mitochondrial ROS (mROS) are basically produced as byproducts of this bioenergetic metabolism
  - Cyt c, cytochrome *c*; MAO, monoamine oxidase; NOX-4, NADPH oxidase 4; VDAC, voltage-dependent anion channel



Nature Reviews | Cardiology

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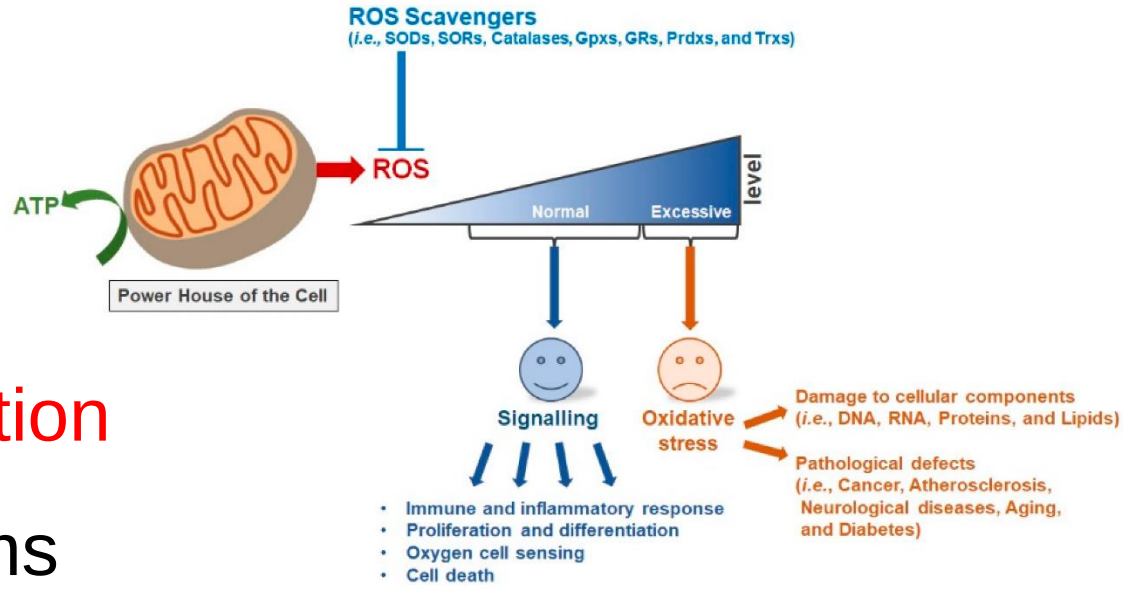
Oxidative stress



Int. J. Mol. Sci. **2019**, *20*(18), 4407

- result of imbalance between

ROS production and antioxidation

- pathological defects in living organisms
  - cancer, atherosclerosis, neurological diseases, aging, and diabetes, damage of cellular components (DNA, RNA, lipids, and proteins)
- non-enzymatic defense:
  - flavonoids, vitamins (A, C, and E), and glutathione
- enzymatic antioxidants:
  - Superoxide dismutase (SOD), superoxide reductase, catalase, glutathione peroxidase, glutathione reductase, peroxiredoxins (Prdxs), and thioredoxins (Trx)

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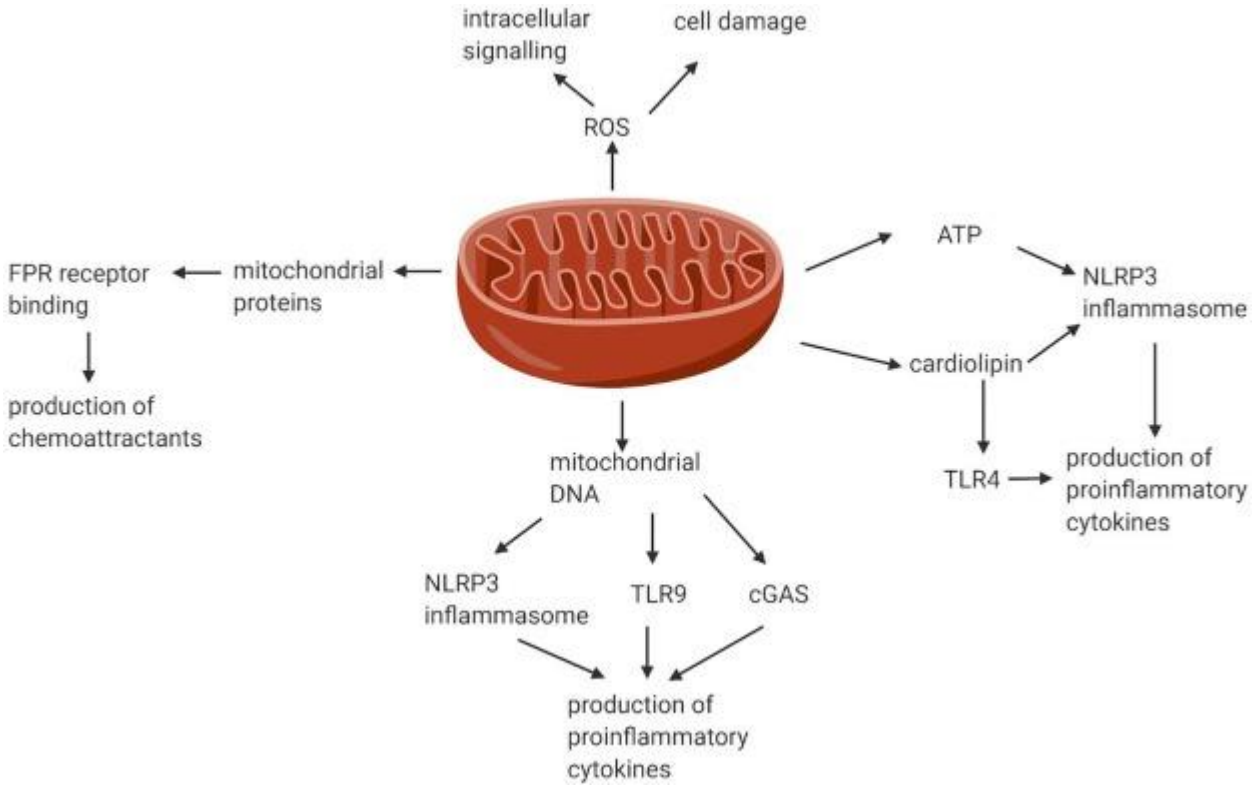
Effect of mitochondria in immune reaction

- mitochondrial DAMPs
  - in extracellular space and circulation.
- mitochondrial proteins
  - FRP receptors - production of chemoattractants.
- mitochondrial ROS
  - intracellular signaling, damage cells.
- mitochondrial ATP and cardiolipin
  - activate the NLRP3 inflammasome or TLR4 - production of pro-inflammatory cytokines.
- mitochondrial DNA
  - activate TLR9, NLRP3 inflammasomes or the cGAS pathway - production of pro-inflammatory cytokines.



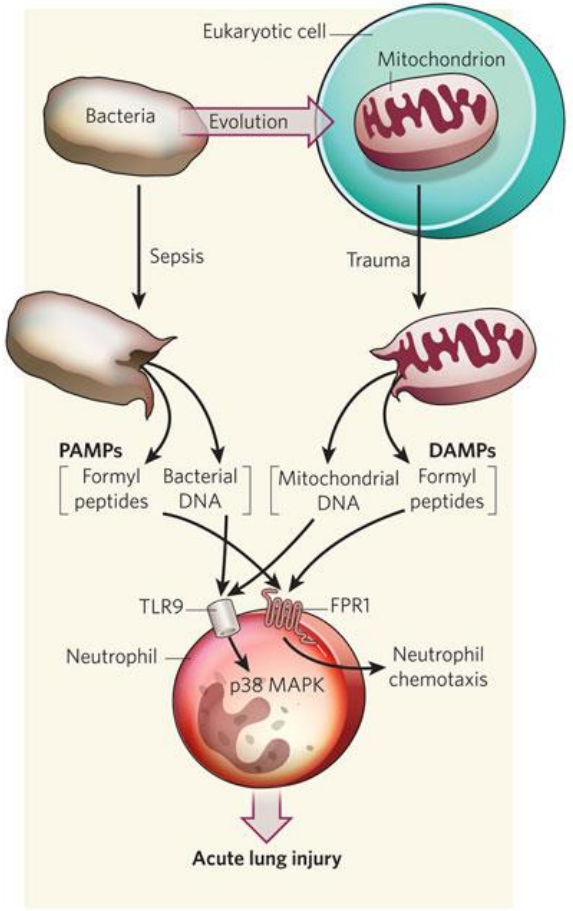
Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease
Volume 1866, Issue 10, 1 October 2020, 165845

MUNI MED

Mitochondria – induction of immune response

- mitochondrial danger associated molecules (DAMPs) that resemble structures of bacterial derived pathogen associated molecular patterns (PAMPs)
  - mitochondrial DAMPS - mitochondrial DNA with hypomethylated CpG motifs, specific lipid present in prokaryotic bacteria and mitochondria, i.e. cardiolipin.
- via DAMPs mitochondria guide the immune response

- mitochondrial DAMPs - negative impact- released by damaged cells, without the presence of an infection - undesired inflammatory response, resulting in tissue damage and organ dysfunction
  - after a trauma

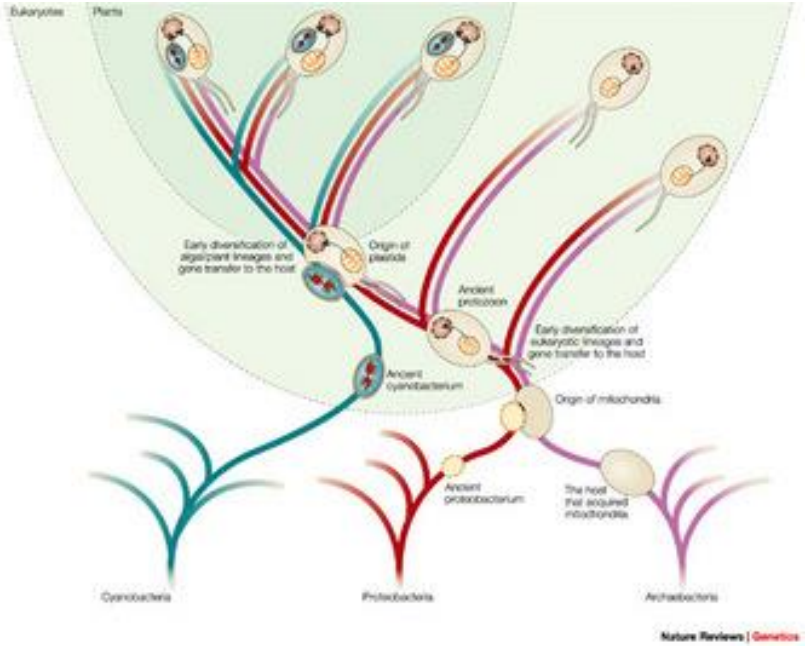


Nature Education 3(9):15

MUNI MED

PRRs as "microbial sensors"

- to detect a set of evolutionarily conserved molecules found in a variety of pathogens - PAMPs, expressed in a wide variety of microorganisms, including those that do not cause disease.
  - In patients with severe infections such as sepsis, PAMPs are the major external "stimulators" of the inflammatory response.
- DAMPs are capable of initiating an inflammatory response similar to that produced by PAMPs, no microbial infections present.
  - DAMPs in SIRS (internal or endogenous "stimulators")

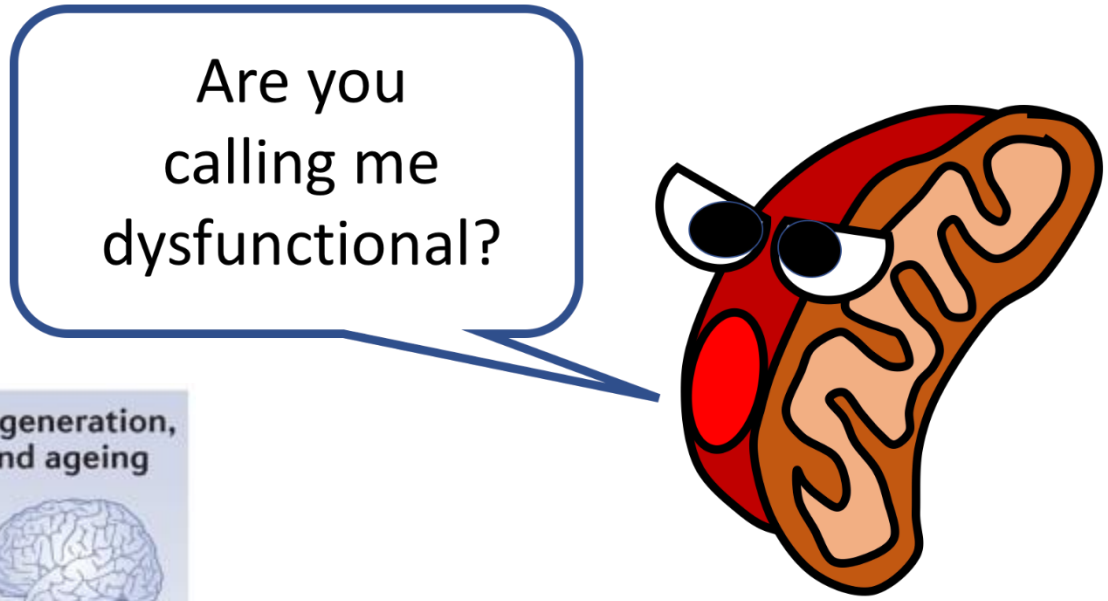


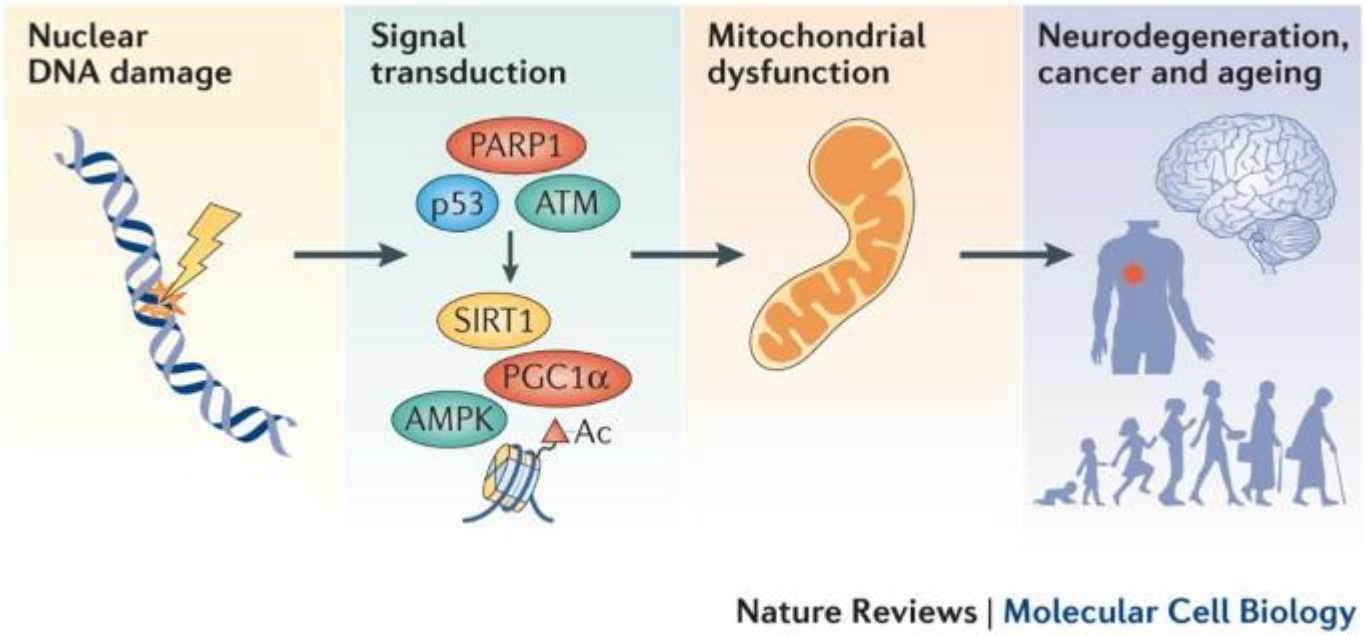
Mitochondria originated following endocytosis of a proteobacteria by another prokaryotic cell.

Nature Reviews Genetics **5,** 123–135

MUNI MED

Mitochondrial dysfunction





Nature Reviews Molecular Cell Biology **volume 17**, pages308–321(2016)

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Mitochondrial dysfunction

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Mitochondrial dysfunction and heart

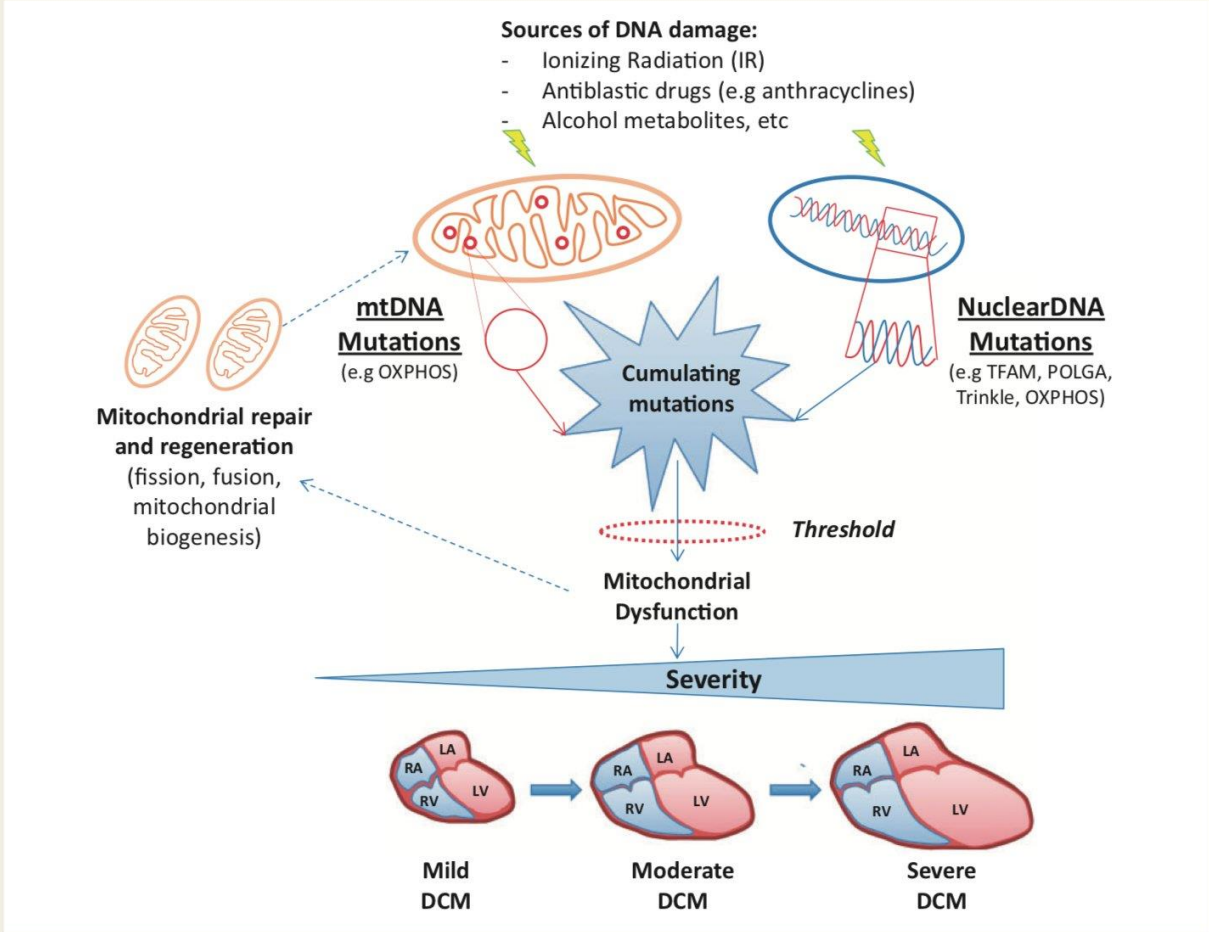


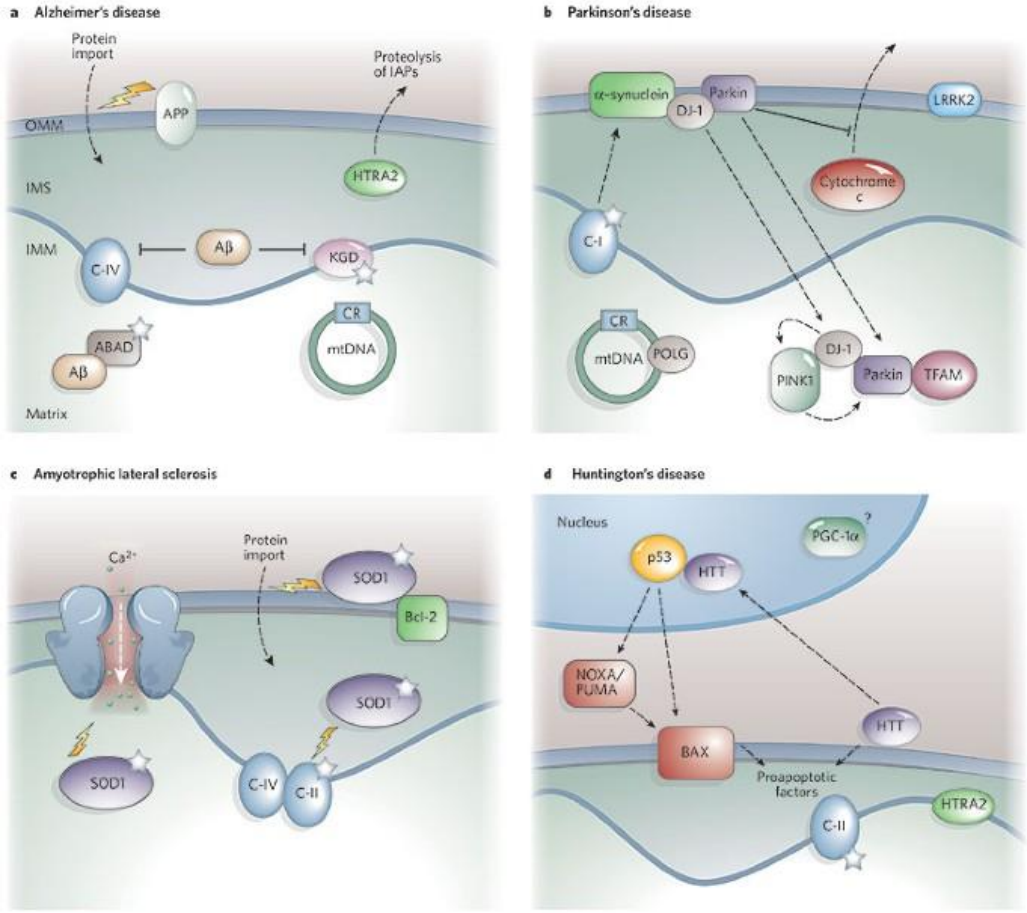
Figure 4 The interaction between DNA defects and failing of mitochondrial repair systems promotes the genesis of mitochondrial DCM. The exposure of cardiomyocytes to ionizing radiation (IR), antiblastic drugs, alcohol metabolites, and many other sources of DNA damage leads to mutations in both mitochondrial and nuclear genes coding for mitochondrial proteins. When the number of gene defects overcomes cellular tolerance, mitochondria can recruit a complex pathway of self-repair and regeneration. As the above mechanisms fail, defects tend to accumulate further, leading at the end to mitochondrial dysfunction.

European Society of Cardiology Journals 23. 10. 2018

MUNI MED

Mitochondrial dysfunction

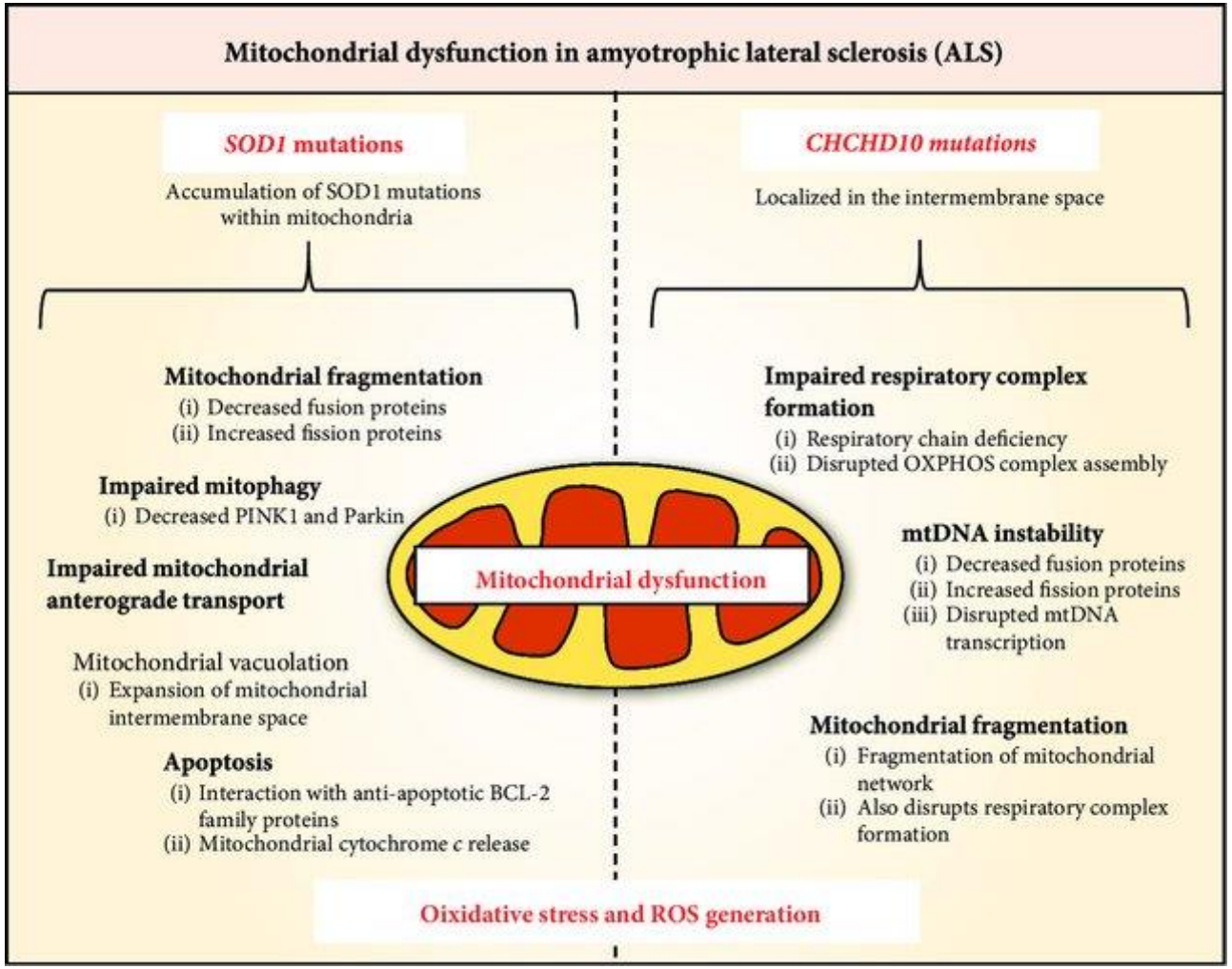
- mutations in mitochondrial DNA and oxidative stress – risk factor for neurodegenerative diseases
  - strong evidence that mitochondrial dysfunction occurs early and acts causally in disease pathogenesis
- disease-specific proteins interact with mitochondria



Nature **volume 443**, pages787–795(2006)

MUNI MED

Mitochondrial dysfunction



Oxidative Medicine and Cellular Longevity 2019

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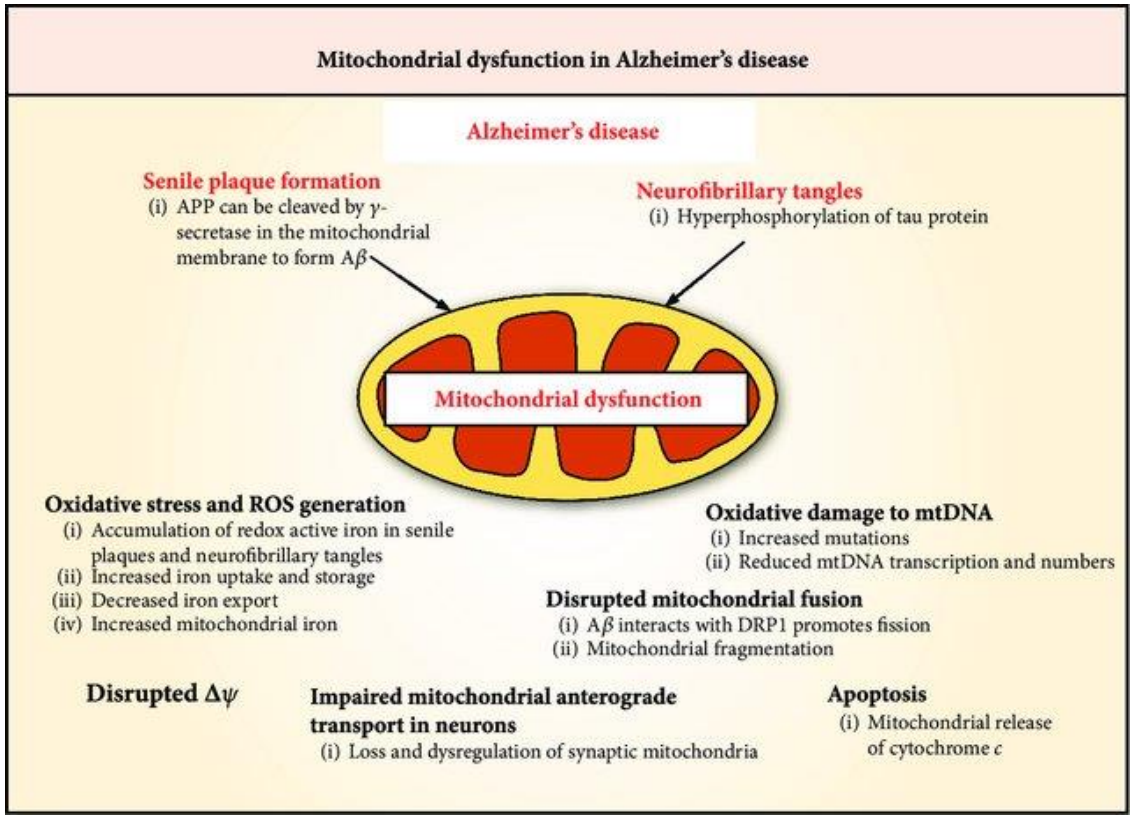
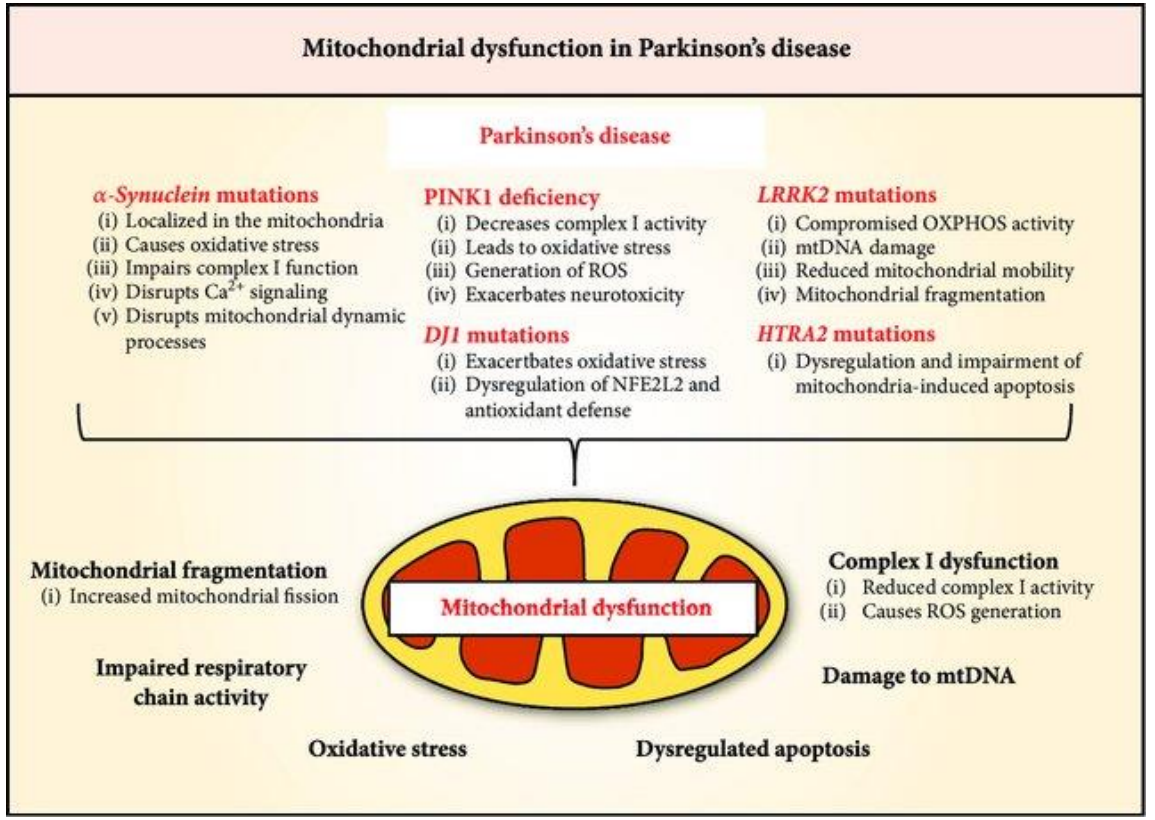
Mitochondrial dysfunction





Oxidative Medicine and Cellular Longevity 2019

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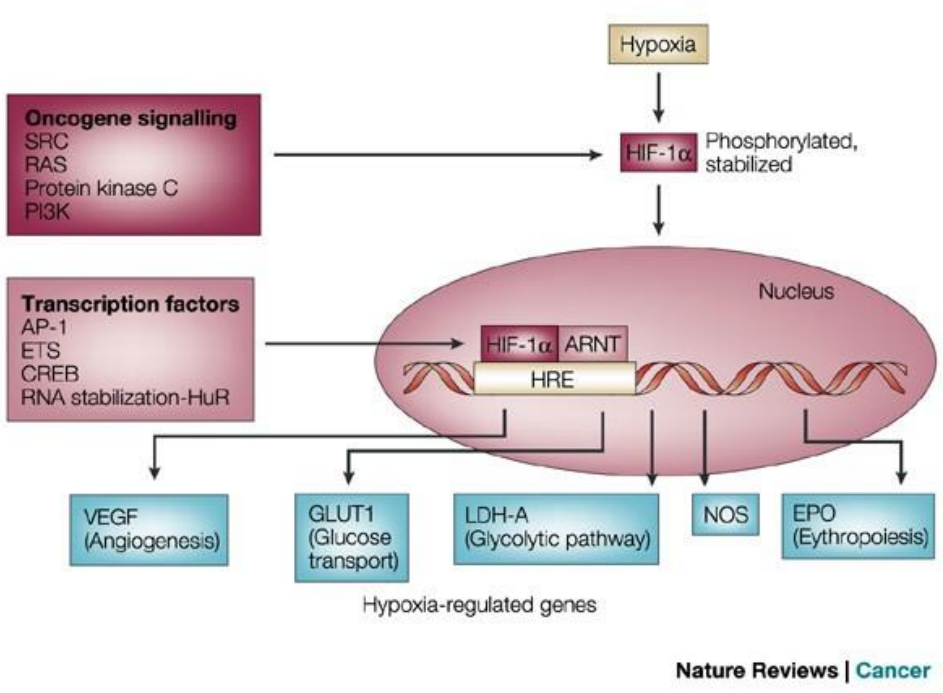
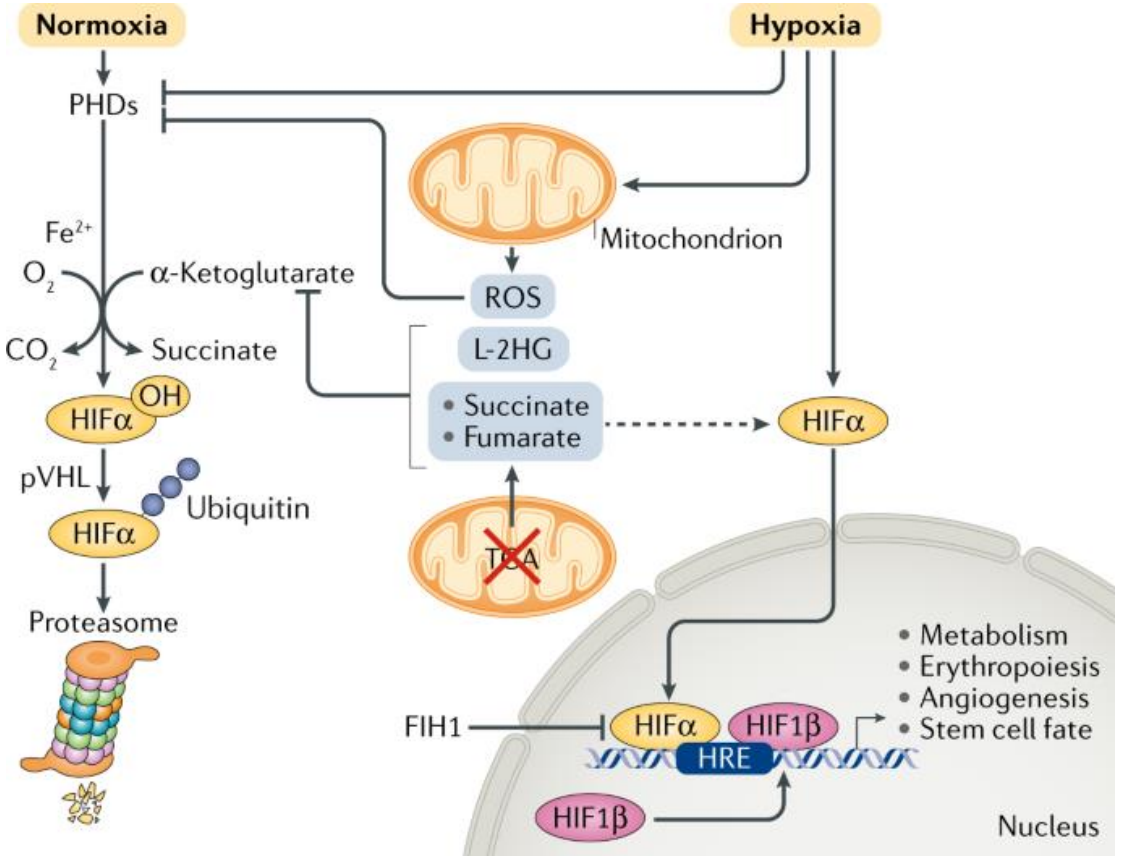
Mitochondria and hypoxia



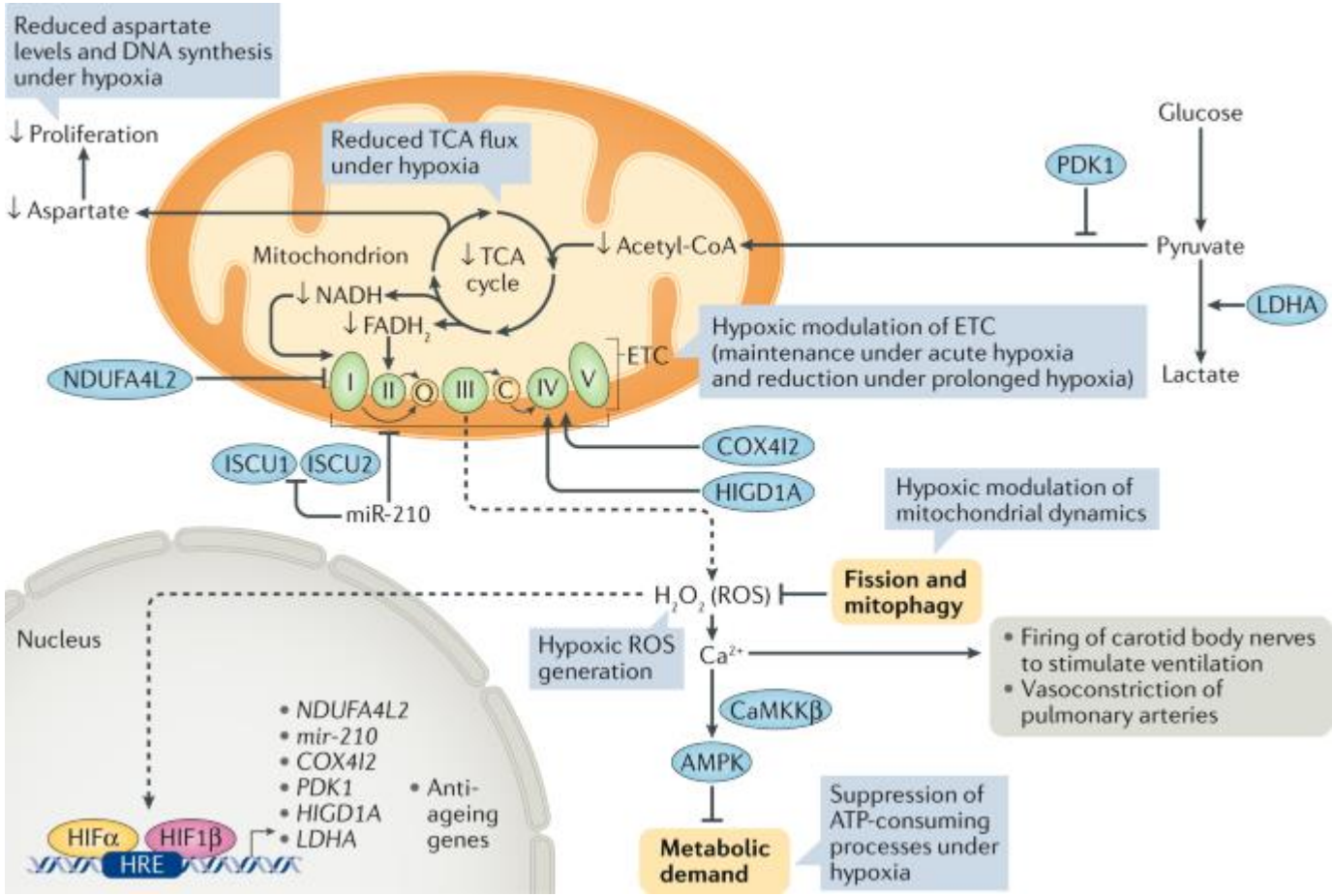


Nature Reviews Cancer **volume 2**, pages38–47(2002)

MUNI MED

Mitochondria and hypoxia

- Acute/chronic
  - decreased flux through the tricarboxylic acid (TCA) cycle
  - activity of the electron transport chain (ETC)
  - hypoxia-induced ROS, …
- low vs. no oxigen



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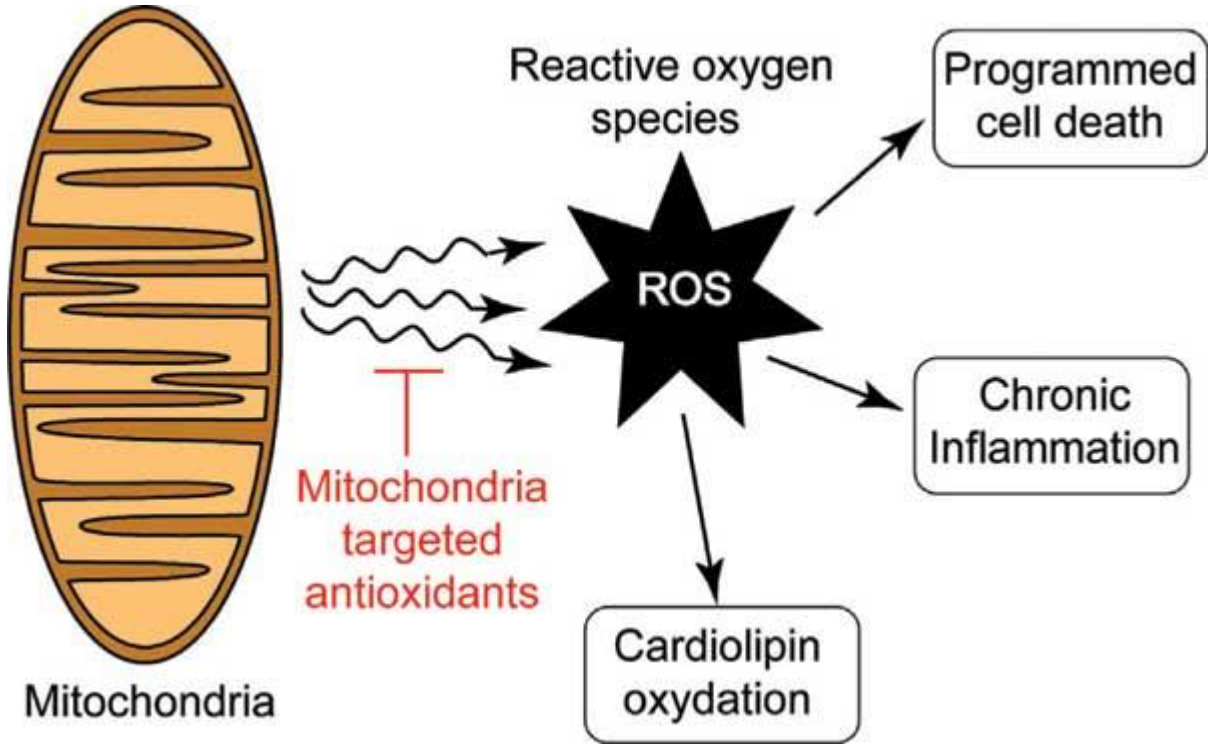
Cell death



Current Aging Science **Volume 10 , Issue 1 , 2017**

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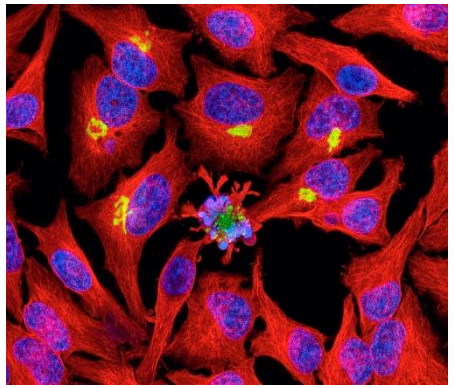
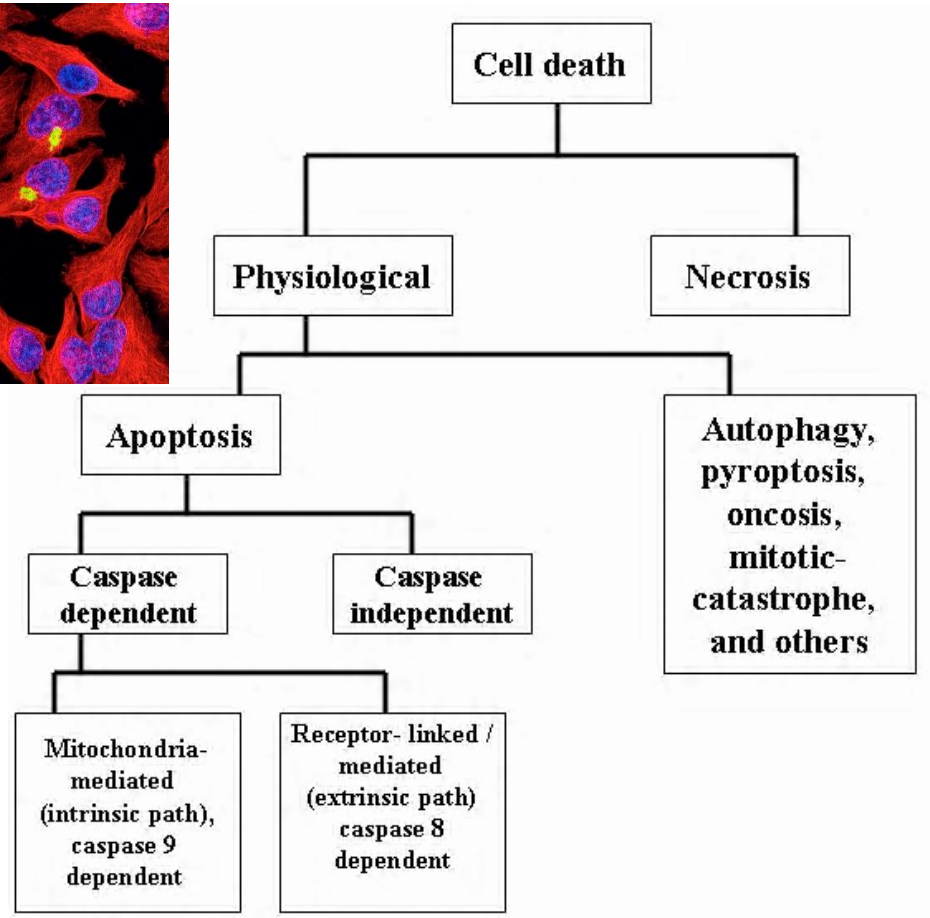
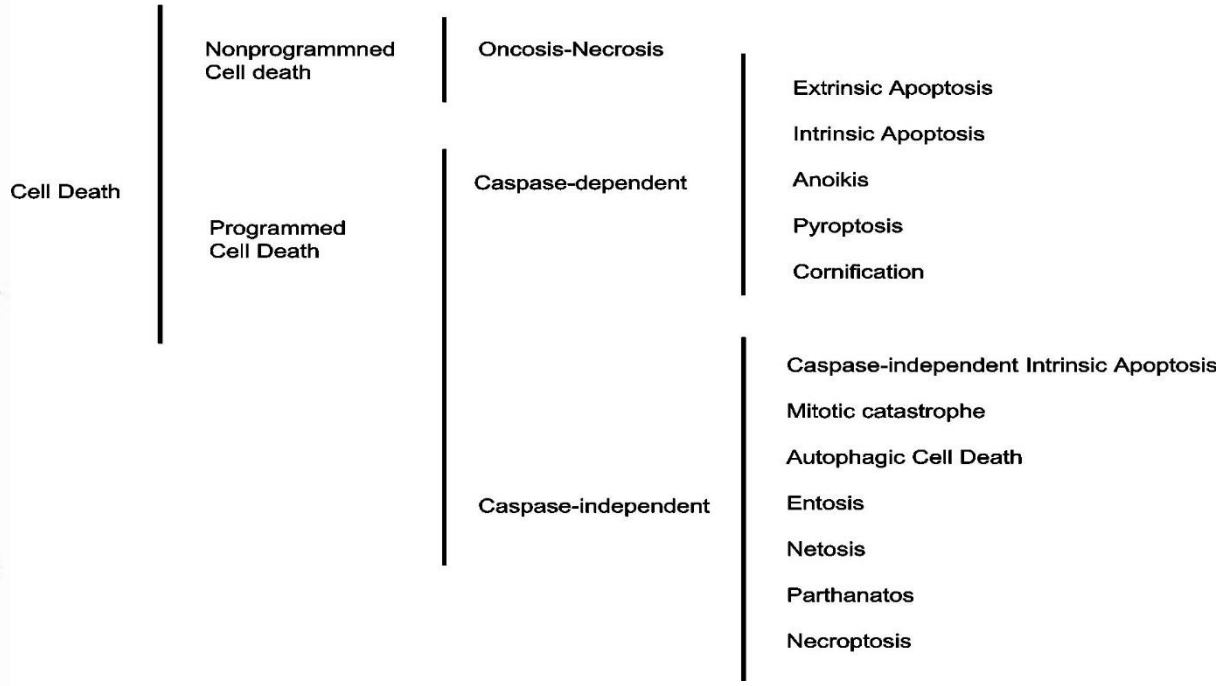
Cell death







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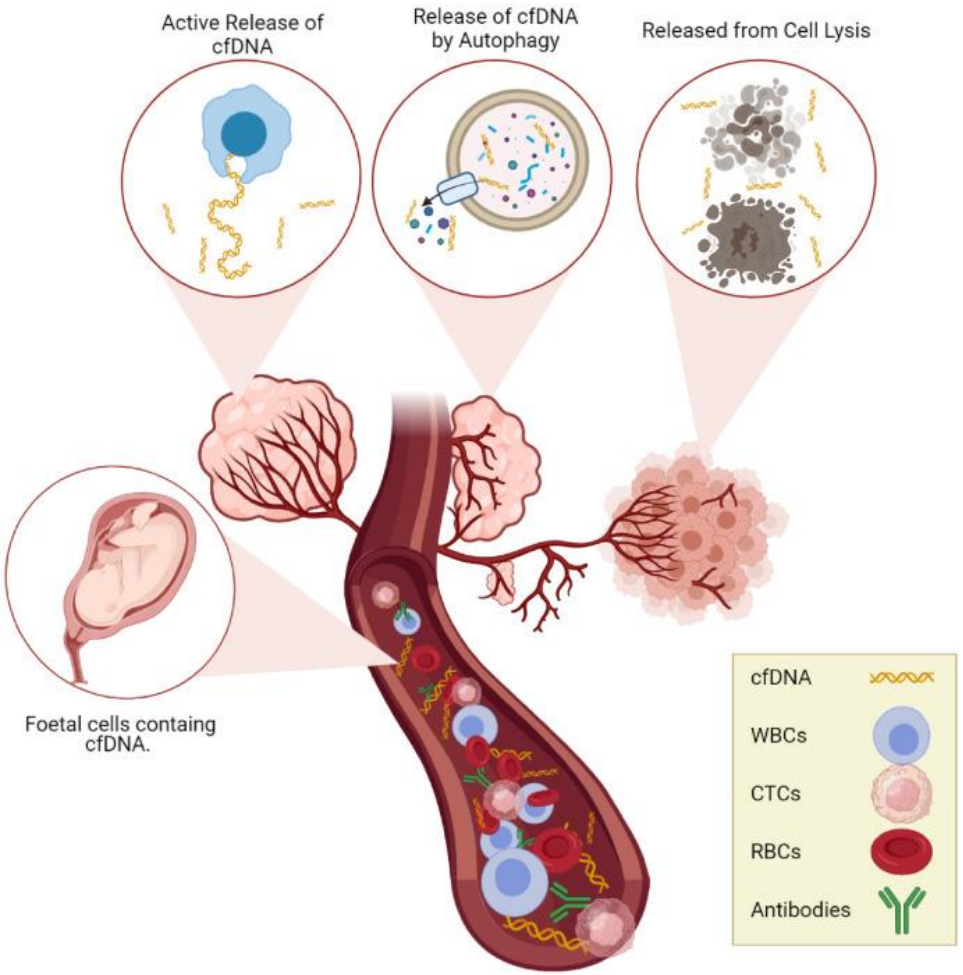
Cell free DNA



- Cellular processes, from cell lysis to DNA release the major sites for cfDNA synthesis.
- cfDNA is often used in its ability to analyze cancer and related diseases in a diagnostic or prognostic aspect
  - present in fetal fluids - an important biomarker, to diagnose abnormalities and as a prenatal analysis - example is Down's Syndrome

Advances in Cancer Biology - Metastasis
Volume 5, October 2022

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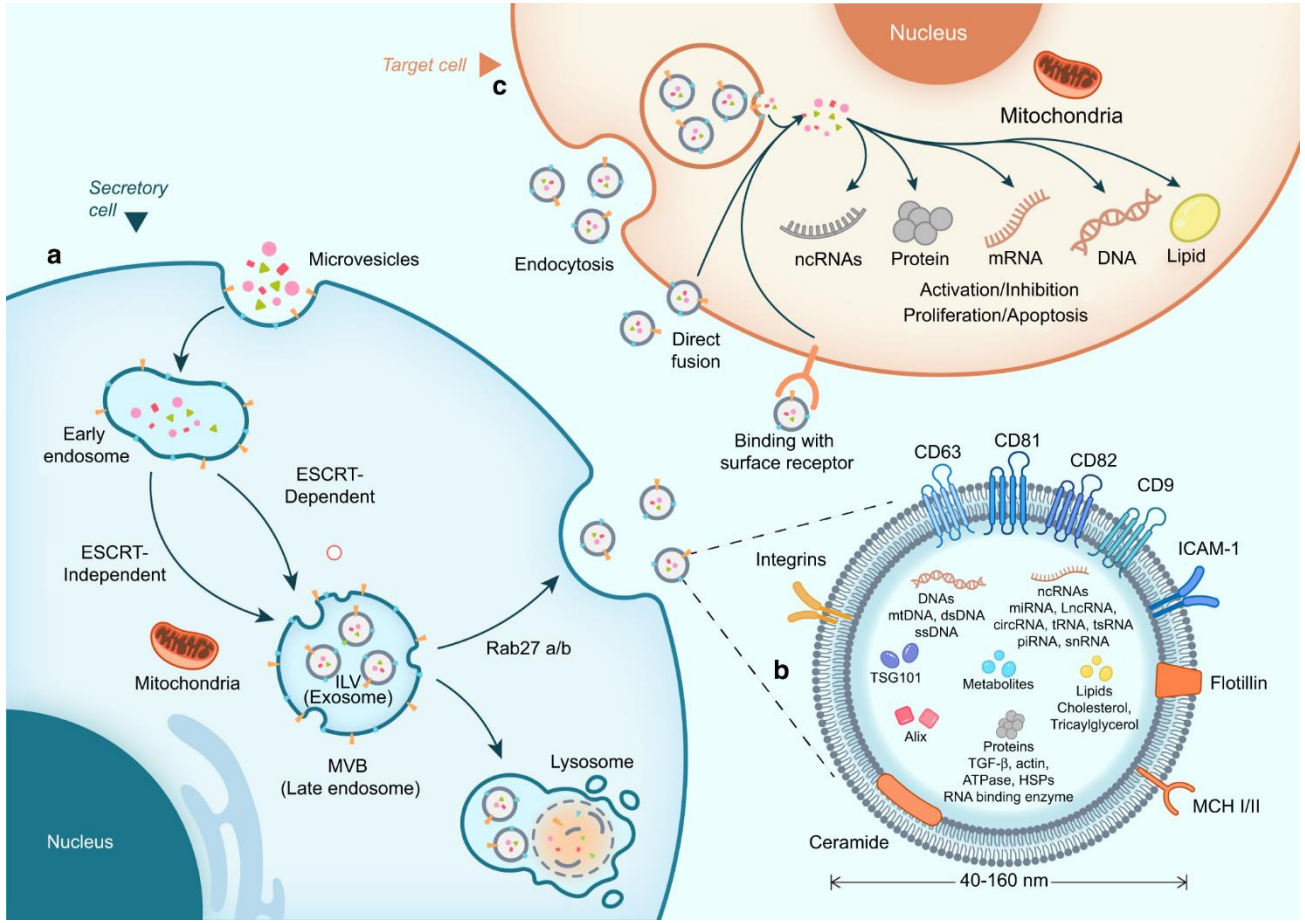
Exosoms

- contain a variety of intrinsic components and variable cargoes, varying from proteins, lipids, metabolites, and DNAs to ncRNAs
- onboard cargoes initiate biological responses and phenotypic changes in recipient cells
  - carrying anabolic/catabolic and anti-/proinflammatory factors
- exosomes serve as a key medium for cell–cell communication



International Journal of Oral Science volume 14, Article number: 40 (2022)

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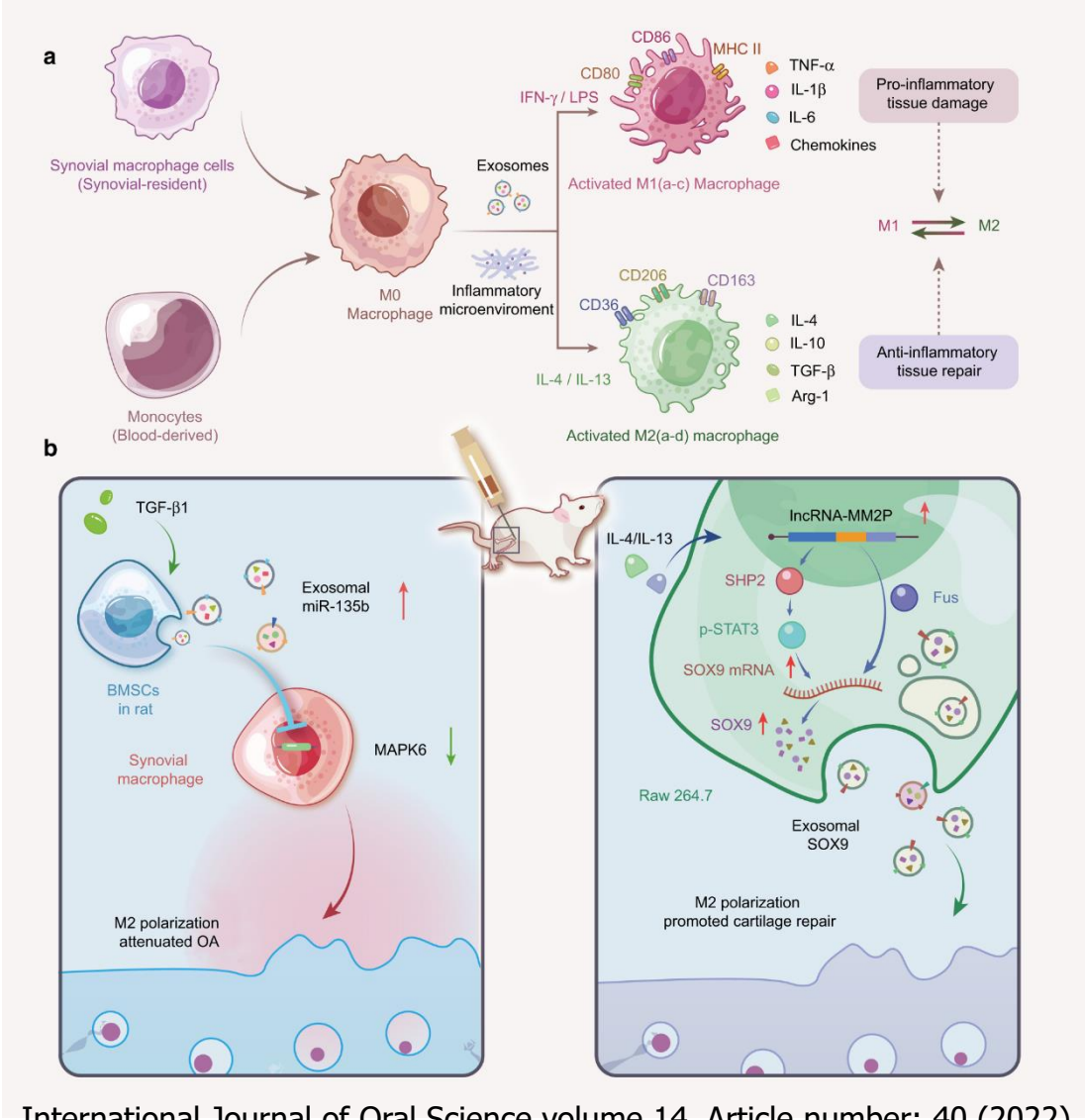
Exosoms in therapy

– progression of OA can be alleviated by facilitating the reprogramming of macrophages from the proinflammatory M1 to the anti-inflammatory M2 subset



International Journal of Oral Science volume 14, Article number: 40 (2022)

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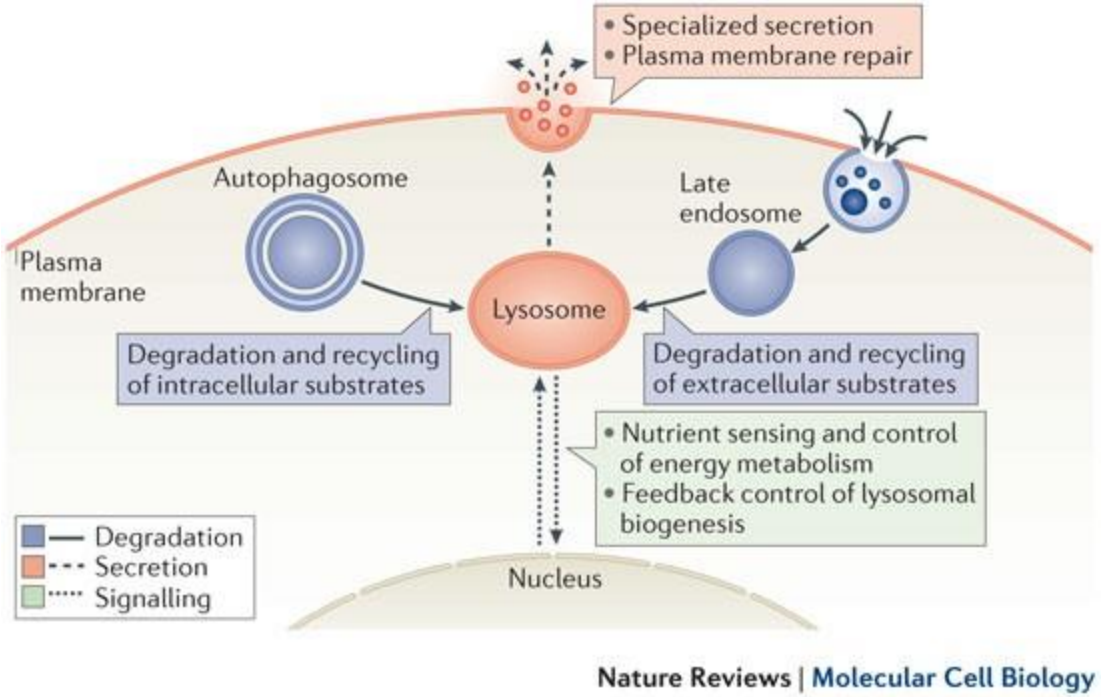
Signals from the lysosome: a control centre for cellular clearance and energy metabolism

- degradation and recycling of cellular waste
- via endocytosis and autophagy
- Lysosomal and autophagy dysfunction
  - lysosomal storage diseases (LSDs) and
  - common neurodegenerative diseases
- defective cellular clearance and accumulation of toxic material

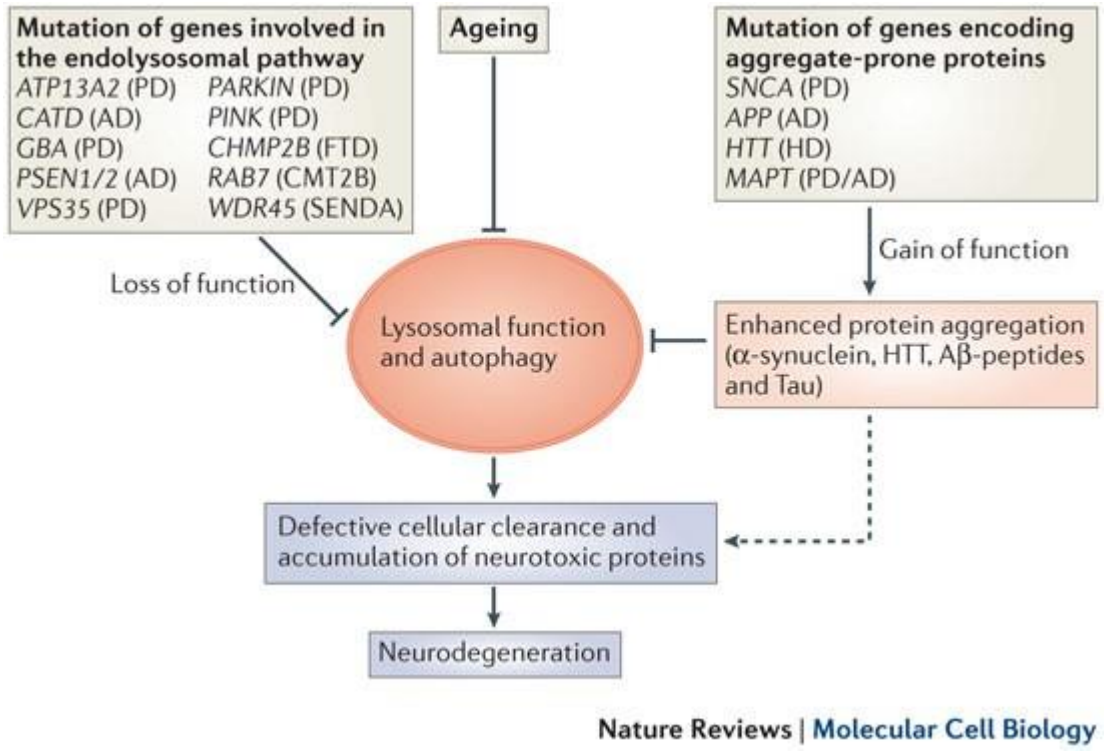


Nature Reviews Molecular Cell Biology **volume 14**, pages283–296(2013)

MUNI MED

Defective cellular clearance in neurodegenerative diseases

- loss-of-function mutations of genes involved in the lysosomal–autophagic pathway
- gain-of-function mutations of aggregate-prone proteins
  - enhanced protein aggregation and impairment of lysosomal–autophagic pathways



Nature Reviews Molecular Cell Biology **volume 14**, pages283–296(2013)

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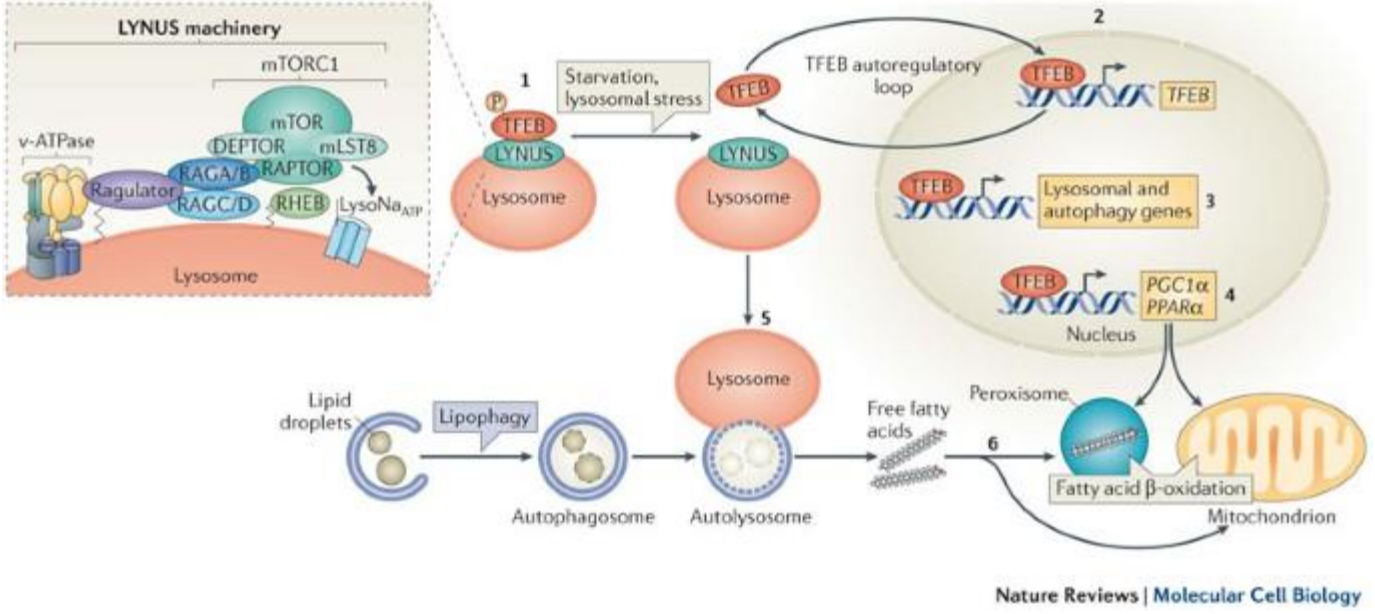
Lysosoms and starvation

- limited nutrient availability and mediates the starvation response by regulating lipid catabolism
- used also by tumor cells



Nature Reviews Molecular Cell Biology **volume 14**, pages283–296(2013)

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Inflammation

Inflammation
Acute fase reaction
Cytokines, chemokines

Inflammation

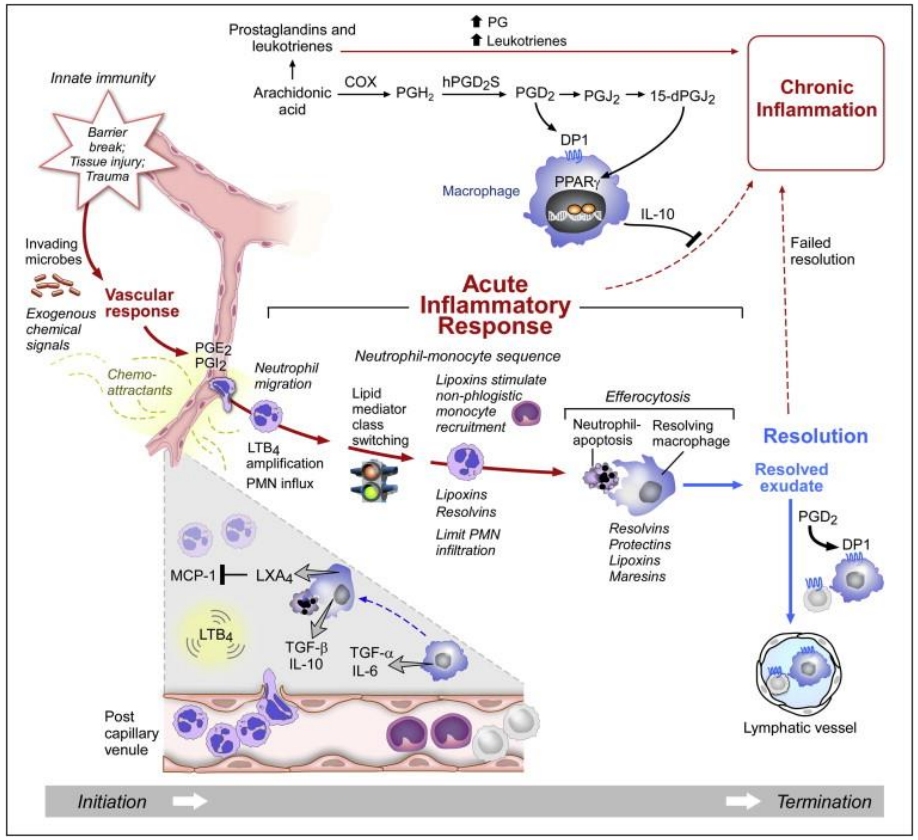
→ Inflammation is the response of living tissue to damage.

→The acute inflammatory response has 3 main functions:

→The affected area is occupied by a transient material called the acute inflammatory exudate. The exudate carries proteins, fluid and cells from local blood vessels into the damaged area to mediate local defences.

→If an infective causative agent (e.g. bacteria) is present in the damaged area, it can be destroyed and eliminated by components of the exudate.

→The damaged tissue can be broken down and partially liquefied, and the debris removed from the site of damage.



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Inflammation

- In all these situations, the inflammatory stimulus will be met by a series of changes in the human body; it will induce production of certain cytokines and hormones, which in turn will regulate haematopoiesis, protein synthesis and metabolism.
- Most inflammatory stimuli are controlled by a normal immune system. The human immune system is divided into two parts which constantly and closely collaborate - the innate and the adaptive immune system.

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Inflammation – innate system

- The innate system reacts promptly without specificity and memory. Phagocytic cells are important contributors in innate reactivity together with enzymes, complement activation and acute phase proteins.

- When phagocytic cells are activated, the synthesis of different cytokines is triggered. These cytokines are not only important in regulation of the innate reaction, but also for induction of the adaptive immune system. There, specificity and memory are the two main characteristics.

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Inflammation – adaptive immune response

– In order to induce a strong adaptive immune response, some lymphocytes must have been educated to recognize the specific antigen on the antigen-presenting cell (APC) in context of self major histocompatibility molecules. The initial recognition will mediate a cellular immune reaction, production of antigen-specific antibodies or a combination of both. Some of the cells, which have been educated to recognize a specific antigen will survive for a long time with the memory of the specific antigen intact, rendering the host "immune" to the antigen.

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Differences between innate (non-specific) and specific (adaptive) immunologic reaction of organism

Non-specific Immunity

- Response is antigen-independent
- There is immediate maximal response
- Non-antigen-specific
- Exposure results in no immunologic memory

Specific Immunity

- Response is antigen-dependent
- There is a lag time between exposure and maximal response
- Antigen-specific
- Exposure results in immunologic memory

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Causes of Inflammation

- infectious inflammatory stimuli (viruses, bacteria, fungi and parasites)
- by non-infectious inflammatory stimuli, as in rheumatoid arthritis and graft-versus host disease
- by tissue necrosis as in cancer
- by burns and toxic influences caused by drugs or radiation

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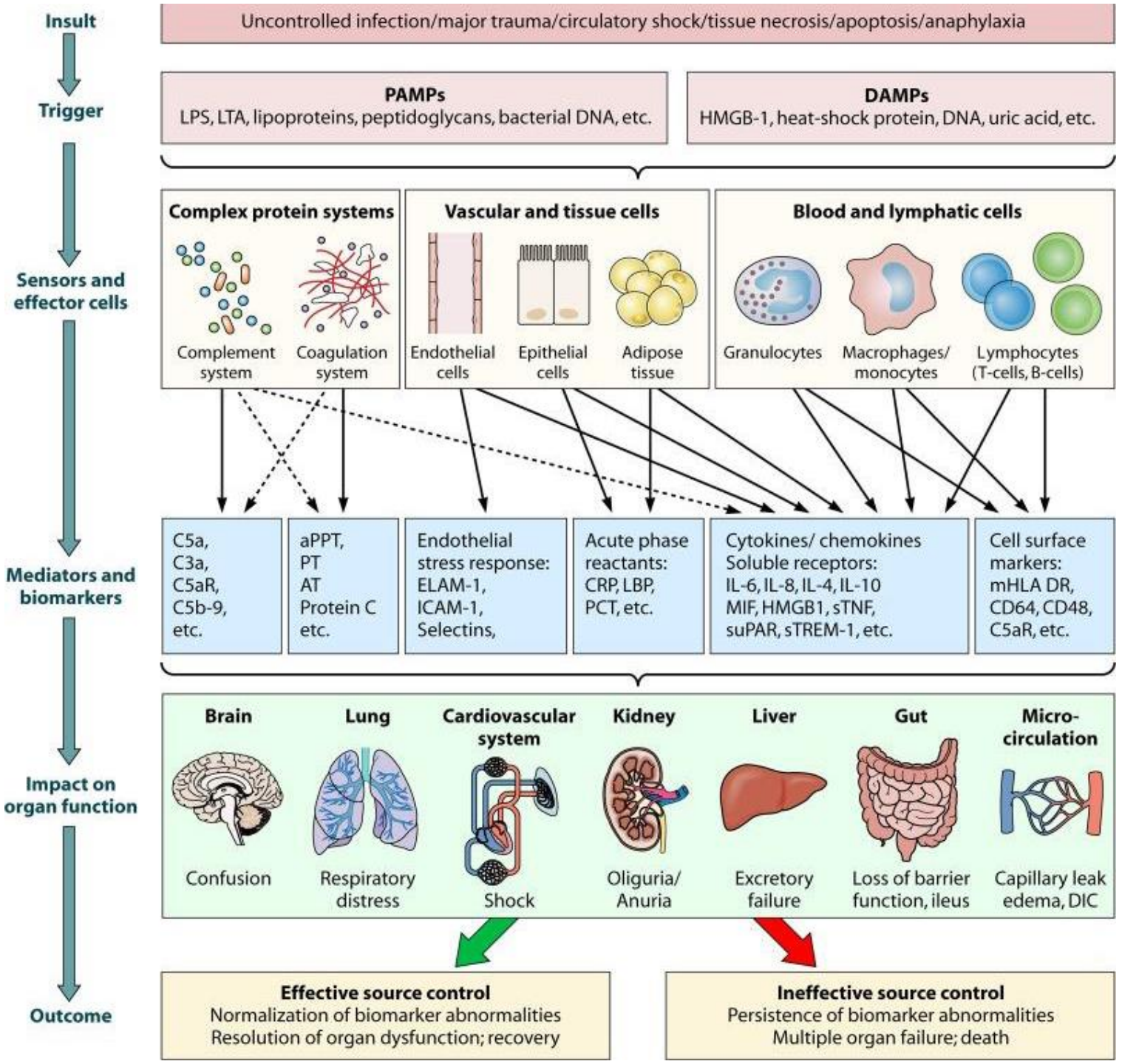

Clin Microbiol Rev. 2012 Oct; 25(4): 609–634.

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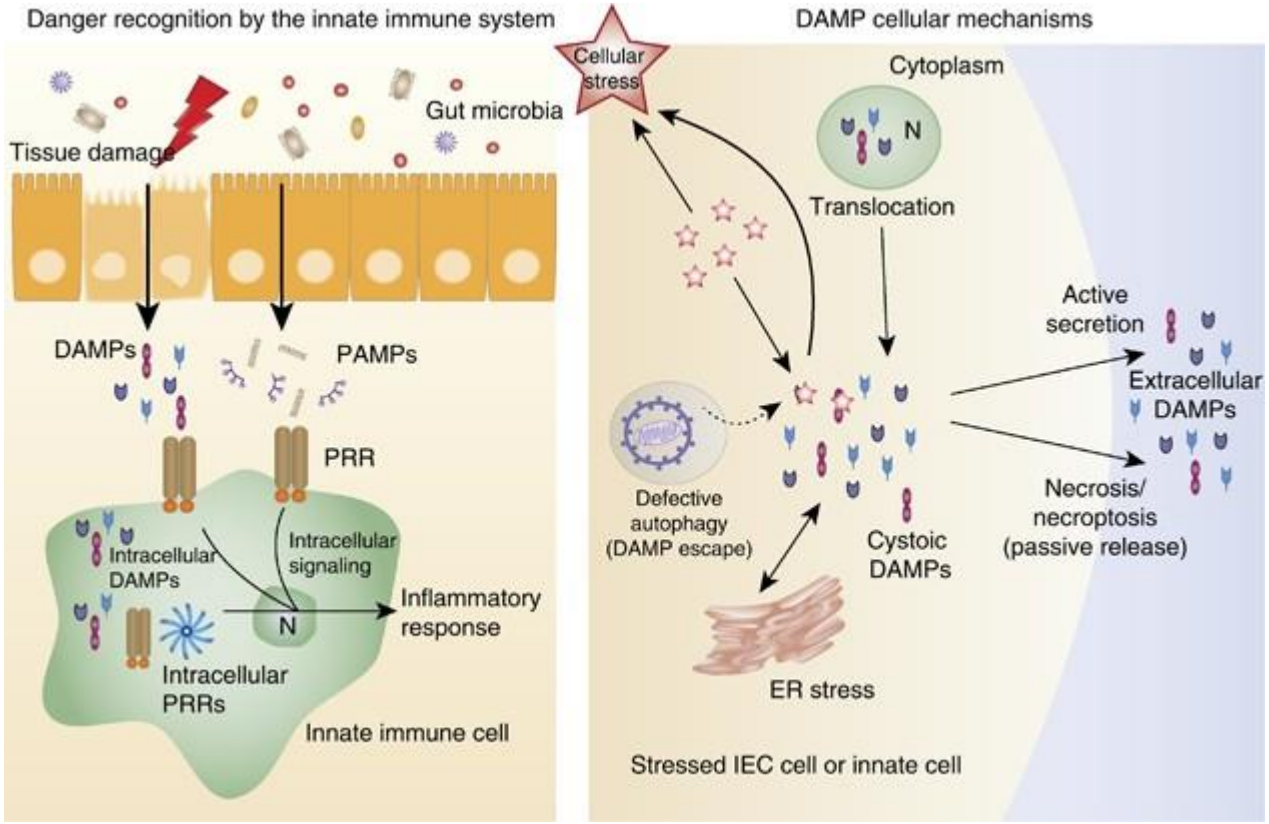
Danger recognition by the innate immune system

- PRRs such as TLR, NLR, and RAGE sense danger associated with infection via recognition of evolutionarily conserved PAMPs on pathogens or sterile injury via recognition of DAMPs.

- Activation of cell surface or intracellular PRRs leads to intracellular signaling and inflammatory responses

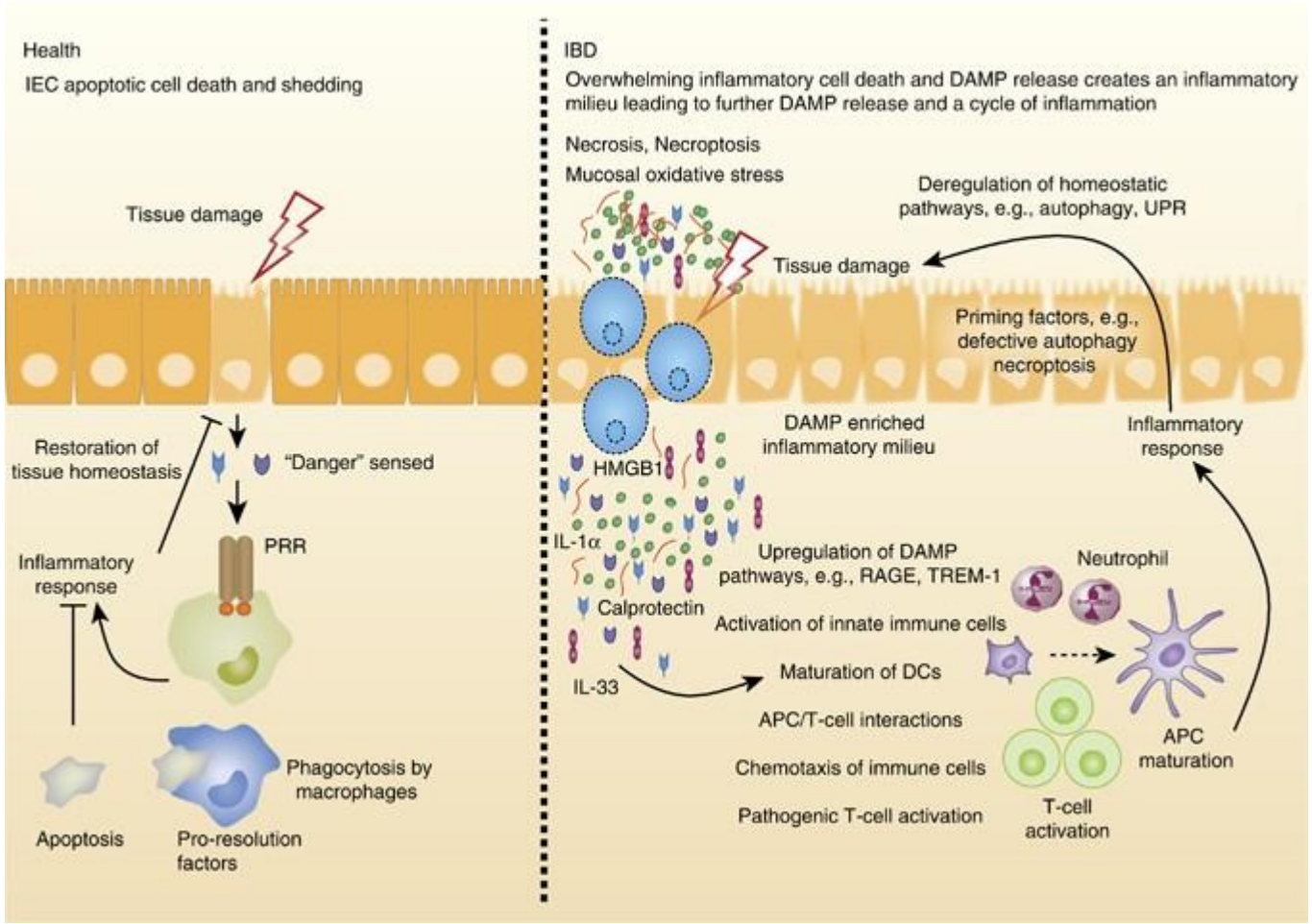


Mucosal Immunology **volume 9**, pages567–582 (2016)

MUNI MED

Contribution of DAMPs to inflammatory response in IBD

– nonapoptotic cell death, mucosal oxidative stress, and deregulation of homeostatic pathways lead to overwhelming release of DAMPs, creating a proinflammatory milieu



Mucosal Immunology **volume 9**, pages567–582 (2016)

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Types of inflammation

- Acute
- Chronic

- Local
- Systemic

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General and local clinical symptoms of the acute phase reaction

Local symptoms	General symptoms
calor	fever
rubor	tachycardia
dolor	hyperventilation
tumor	tiredness
functio laesa	Loss of appetite

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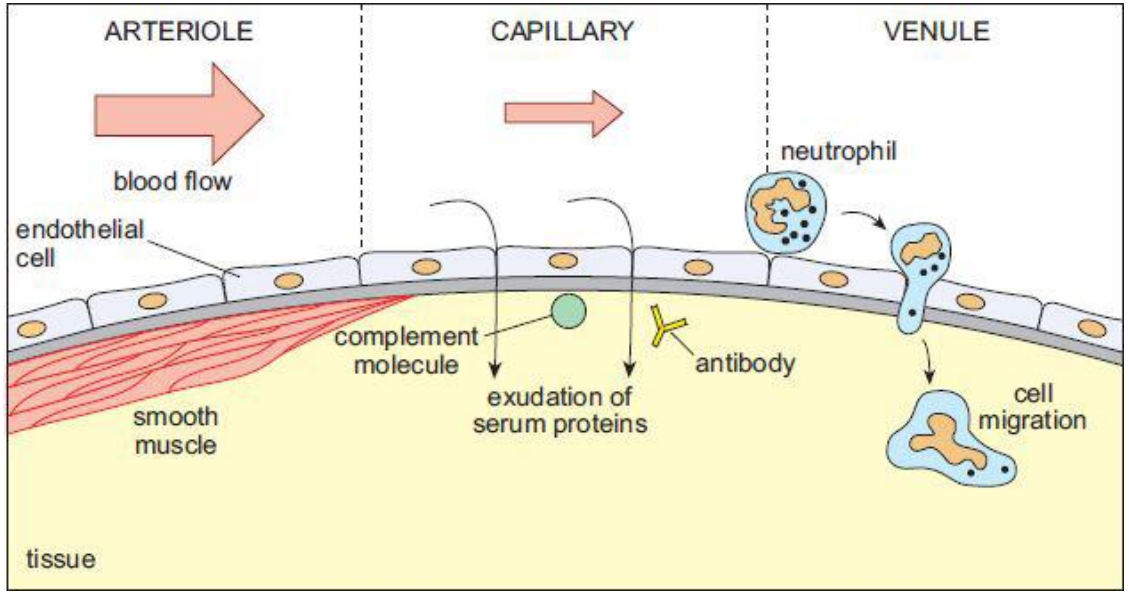
Local Inflammation

The acute inflammatory response involves three processes:

- changes in vessel caliber (= vasodilation) and, consequently, slower blood flow
- increased vascular permeability and formation of the fluid exudate
- formation of the cellular exudate by emigration of the neutrophil polymorphs into the extravascular space.



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Early Stages

The steps involved in the acute inflammatory response are:

- Small blood vessels adjacent to the area of tissue damage initially become dilated with increased blood flow, then flow along them slows down.
- Endothelial cells swell and partially retract so that they no longer form a completely intact internal lining.
- The vessels become leaky, permitting the passage of water, salts, and some small proteins from the plasma into the damaged area (exudation). One of the main proteins to leak out is fibrinogen.
- Circulating neutrophil polymorphs initially adhere to the swollen endothelial cells (margination), then actively migrate through the vessel basement membrane (emigration), passing into the area of tissue damage.
- Later, small number of blood monocytes (macrophages) migrate in a similar way, as do lymphocytes.

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Systemic manifestation of inflammation

- **Increase of body temperature (fever)**
- **Acute phase reaction**

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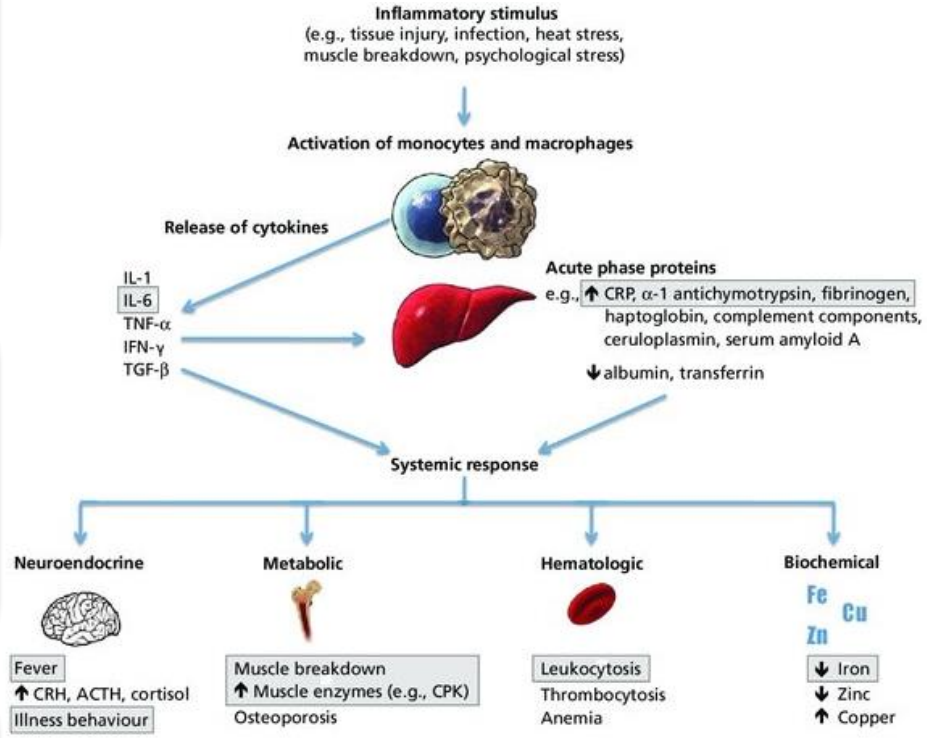
Acute phase reaction

The acute phase reaction is the body's first-line inflammatory defense system, functioning without specificity and memory, and in front of, and in parallel with, the adaptive immune system.

In the acute phase reaction, several biochemical, metabolic, hormonal and cellular changes take place in order to fight the stimulus and re-establish a normal functional state in the body.

An **increase in the number of granulocytes** will increase the phagocytotic capacity, an increase in scavengers will potentiate the capability to neutralize free oxygen radicals, and an increase in metabolic rate will increase the energy available for cellular activities, despite a reduced food intake.

Some of these changes can explain the symptoms of an acute phase reaction, which are typically fever, tiredness, loss of appetite and general sickness, in addition to local symptoms from the inducer of the acute phase.



Canadian Medical Association Journal 182(18):E834-8

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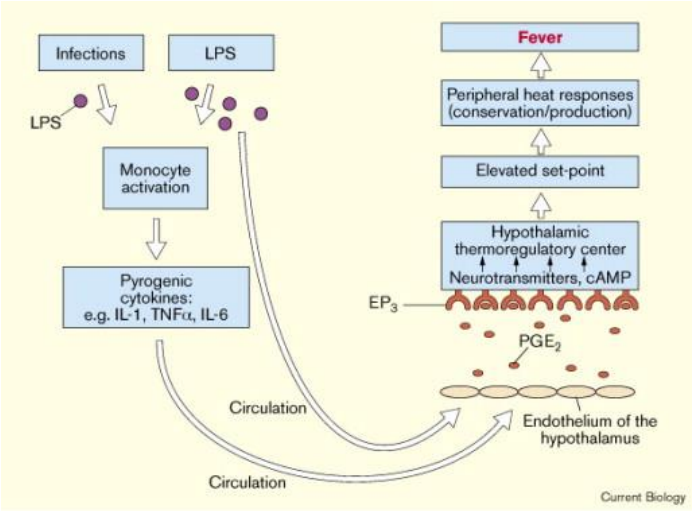
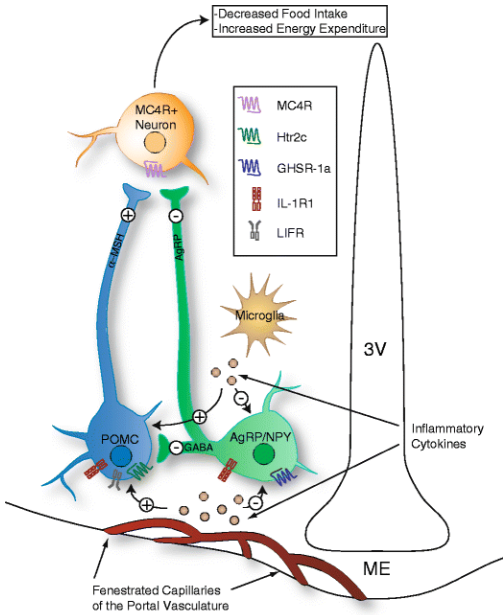
Systemic effects of acute/chronic inflammation



→ Pyrexia

Polymorphs and macrophages produce compounds known as endogenous pyrogens, which act on the hypothalamus to set the thermoregulatory mechanisms at a higher temperature. Release of endogenous pyrogen is stimulated by phagocytosis, endotoxins and immune complexes.



Mol Cancer Res; 11(9); 967–72. ©2013 AACR.

→ Constitutional symptoms

Constitutional symptoms include malaise, anorexia and nausea. Weight loss is common when there is extensive chronic inflammation.

→ Local or systemic lymph node enlargement commonly accompanies inflammation, while splenomegaly is found in certain specific infections (e.g. malaria, infectious mononucleosis).

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Systemic effects of inflammation

Haematological changes

- Increased erythrocyte sedimentation rate. An increased erythrocyte sedimentation rate is a non-specific finding in many types of inflammation.

- Leukocytosis.
  - Neutrophilia occurs in pyogenic infections and tissue destruction;
  - eosinophilia in allergic disorders and parasitic infection;
  - lymphocytosis in chronic infection (e .g. tuberculosis), many viral infections and in whooping cough; and
  - monocytosis occurs in infectious mononucleosis and certain bacterial infections (e.g. tuberculosis, typhoid).

- Anaemia.
  - blood-loss in the inflammatory exudate (e.g. in ulcerative colitis),
  - haemolysis (due to bacterial toxins), and
  - 'the anemia of chronic disorders' due to toxic depression of the bone marrow.

Amyloidosis

- Longstanding chronic inflammation (for example, in rheumatoid arthritis, tuberculosis and bronchiectasis), by elevating serum amyloid A protein (SAA), may cause amyloid to be deposited in various tissues resulting in secondary (reactive) amyloidosis.

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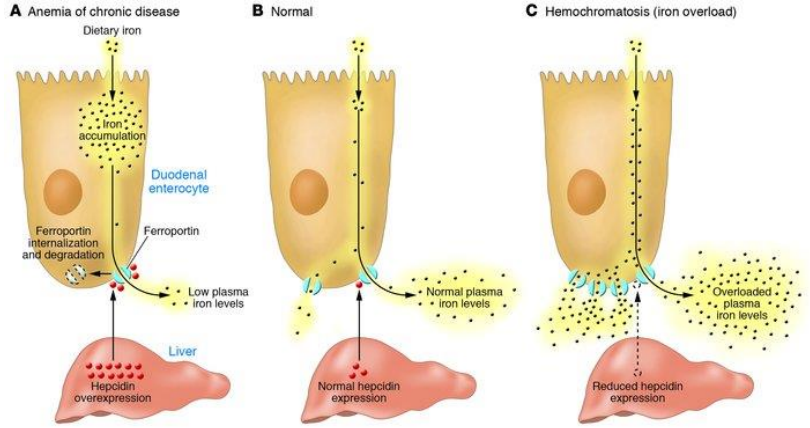
Difference between anaemia of chronic disease and iron-deficiency anaemia



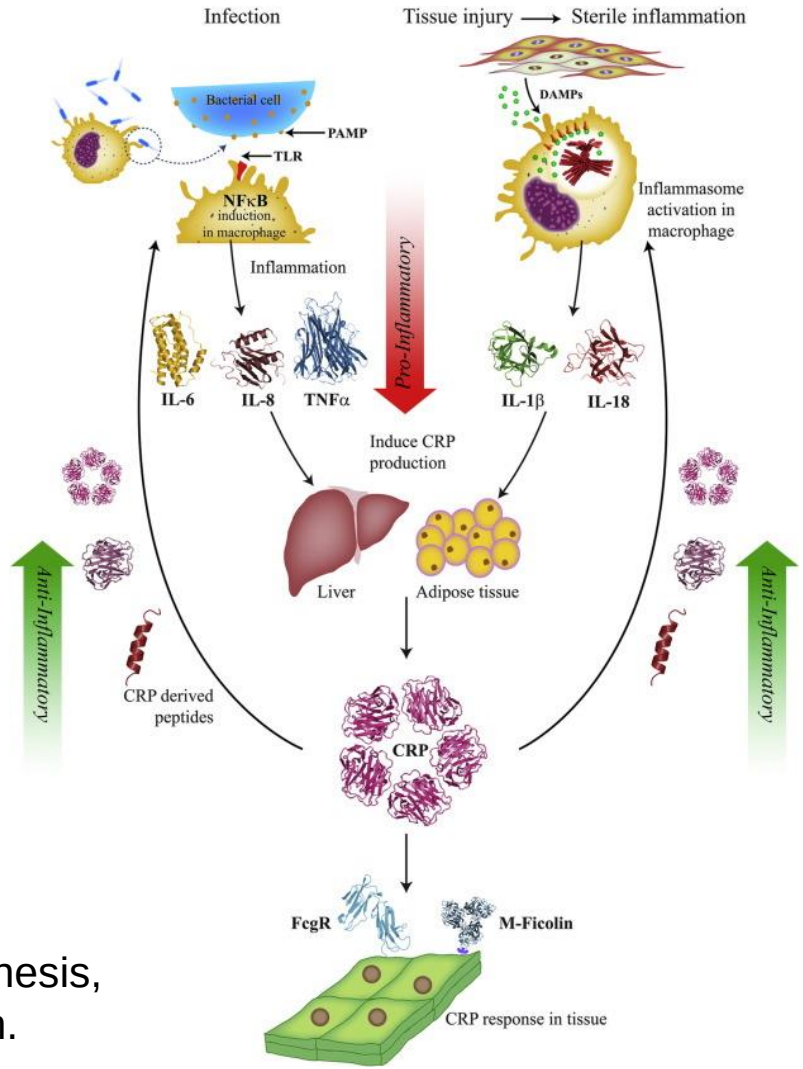
J Clin Invest. 2007;117(7):1755-1758. https://doi.org/10.1172/JCI32701.

	Anemia of Chronic Diseases	Iron Deficiency Anemia
Seru Iron	Reduced	Reduced
Transferrin	Reduced to normal	Increased
Transferrin Saturation	Reduced	Reduced
Ferritin	Normal to increased	Reduced
Soluble transferrin receptor	Normal	Increased
Cytokine level	Increased	Normal
Hepcidin	Increased	Reduced
Bone marrow iron stores	Normal to increased	Reduced
Ery	Normal, microcytes	Microcytes

Acute phase proteins

- Induction of the acute phase reaction - changes in synthesis of many proteins in the liver
  - measured in plasma.

- Regulation of protein synthesis - at the level of both transcription (DNA, RNA) and translation to protein.
  - The cells have intricate systems for up- and down-regulation of protein synthesis, initiated by a complex system of signals induced in the acute phase reaction.



Biotechnology Advances Volume 34, Issue 3, May–June 2016

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Acute phase proteins

Function related to

- limiting the negative effects of the acute phase stimulus

or

- repair of inflammatory induced damage.

Examples are enzyme inhibitors limiting the negative effect of enzymes released from neutrophils, scavengers of free oxygen radicals, increase in some transport proteins and increased synthesis and activity of the cascade proteins such as coagulation and complement factors.
The protein synthesis may be upregulated even if plasma levels are normal, due to increased consumption of acute phase proteins.

TABLE 1. HUMAN ACUTE-PHASE PROTEINS.

Proteins whose plasma concentrations increase
Complement system
C3
C4
C9
Factor B
C1 inhibitor
C4b-binding protein
Mannose-binding lectin
Coagulation and fibrinolytic system
Fibrinogen
Plasminogen
Tissue plasminogen activator
Urokinase
Protein S
Vitronectin
Plasminogen-activator inhibitor 1
Antiproteases
α_1-Protease inhibitor
α_1-Antichymotrypsin
Pancreatic secretory trypsin inhibitor
Inter-α-trypsin inhibitors
Transport proteins
Ceruloplasmin
Haptoglobin
Hemopexin
Participants in inflammatory responses
Secreted phospholipase A_2
Lipopolysaccharide-binding protein
Interleukin-1–receptor antagonist
Granulocyte colony-stimulating factor
Others
C-reactive protein
Serum amyloid A
α_1-Acid glycoprotein
Fibronectin
Ferritin
Angiotensinogen
Proteins whose plasma concentrations decrease
Albumin
Transferrin
Transthyretin
α_2-HS glycoprotein
Alpha-fetoprotein
Thyroxine-binding globulin
Insulin-like growth factor I
Factor XII

N Engl J Med 1999; 340:448-454
DOI: 10.1056/NEJM199902113400607

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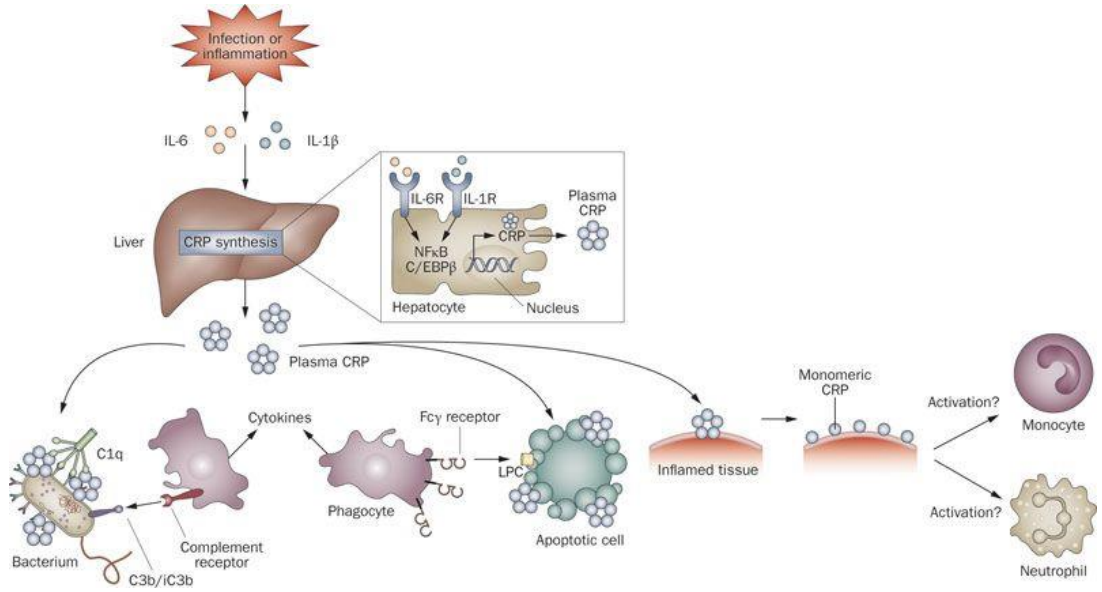
Function	Positive acute phase protein	Increase up to
Protease inhibitors	Alfa 1-antitrypsin Alfa 1-antichymotrypsin	4 x 6 x
Coagulation proteins (serin proteinases)	fibrinogen prothrombin factor VIII plasminogen	8 x
Complement factors	C1s C2b C3, C4, C5 C9 C5b	2 x
Transport proteins	haptoglobin hemopexin ferritin	8 x 2 x 4 x
Scavenger proteins	ceruloplasmin	4 x
Others	alfa1-acid glycoprotein (orosomukoid) serum amyloid A protein C-reactive protein	4 x 1000 x 1000 x

Biochemistry and physiology of the acute phase reaction

– **CRP** is a major acute phase protein acting mainly through Ca2+-dependent binding to, and clearance of, different target molecules in proteins, having evolved almost unchanged from primitive to advanced species.



Nature Reviews Rheumatology **volume 7**, pages282–289 (2011)

microbes, cell debris and cell nuclear material.

In an acute phase reaction there may be a more than 1000-fold increase in the serum concentration of CRP. CRP is regarded as an important member of the family of acute phase, having evolved almost unchanged from primitive to advanced species.

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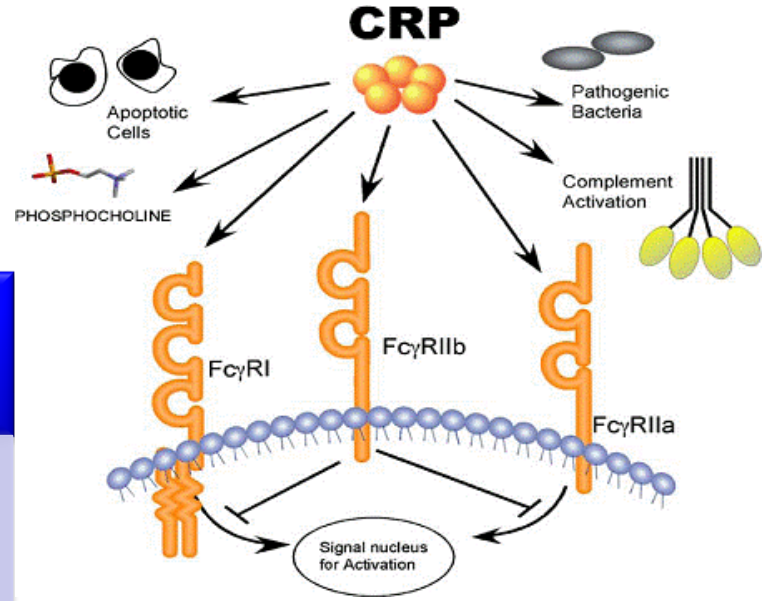
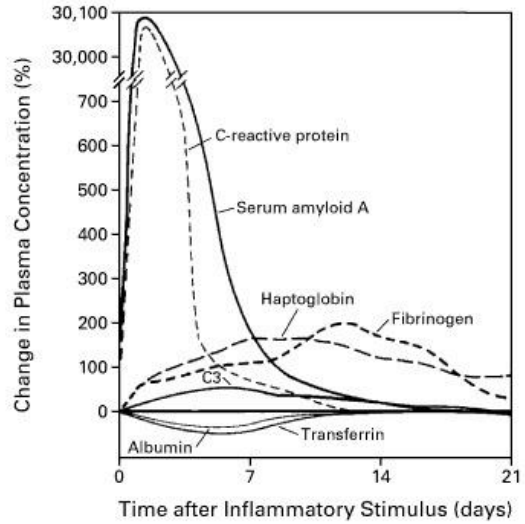
C-reactive protein

Most functions of CRP are easily understood in the context of the body's defenses against infective agents.

- The bacteria are opsonized by CRP and increased phagocytosis is induced.
- CRP activates complement with the split product being chemotactic, increasing the number of phagocytes at the site of infection. Enzyme inhibitors protect surrounding tissue from the damage of enzymes released from the phagocytes.
- CRP binds to chromatin from dead cells and to cell debris which are cleared from the circulation by phagocytosis, either directly or by binding to Fc-, C3b- or CRP-specific receptors. Platelet aggregation is inhibited, decreasing the possibility of thrombosis.
- CRP binds to low density lipoprotein (LDL) and may clear LDL from the site of atherosclerotic plaques by binding to cell surface receptors on phagocytic cells.





N Engl J Med 1999; 340:448-454
DOI: 10.1056/NEJM199902113400607

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Biologically active products of complement activation

Chemotactic factors

C5a and MAC (membrane attack complex C5b67) are both chemotactic. C5a is also a potent activator of neutrophils, basophils and macrophages and causes induction of adhesion molecules on vascular endothelial cells.

Opsonins

C3b and C4b in the surface of microorganisms attach to C-receptor (CR1) on phagocytic cells and promote phagocytosis.

Other biologically active products of C activation

Degradation products of C3 (iC3b, C3d and C3e) also bind to different cells by distinct receptors and modulate their function.

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Biologically active products of complement activation

Activation of complement results in the production of several biologically active molecules which contribute to resistance, anaphylaxis and inflammation.

Kinin production

C2b generated during the classical pathway of C activation is a prokinin which becomes biologically active following enzymatic alteration by plasmin.

Anaphylotoxins

C4a, C3a and C5a (in increasing order of activity) are all anaphylatoxins, which cause basophil/mast cell degranulation and smooth muscle contraction.

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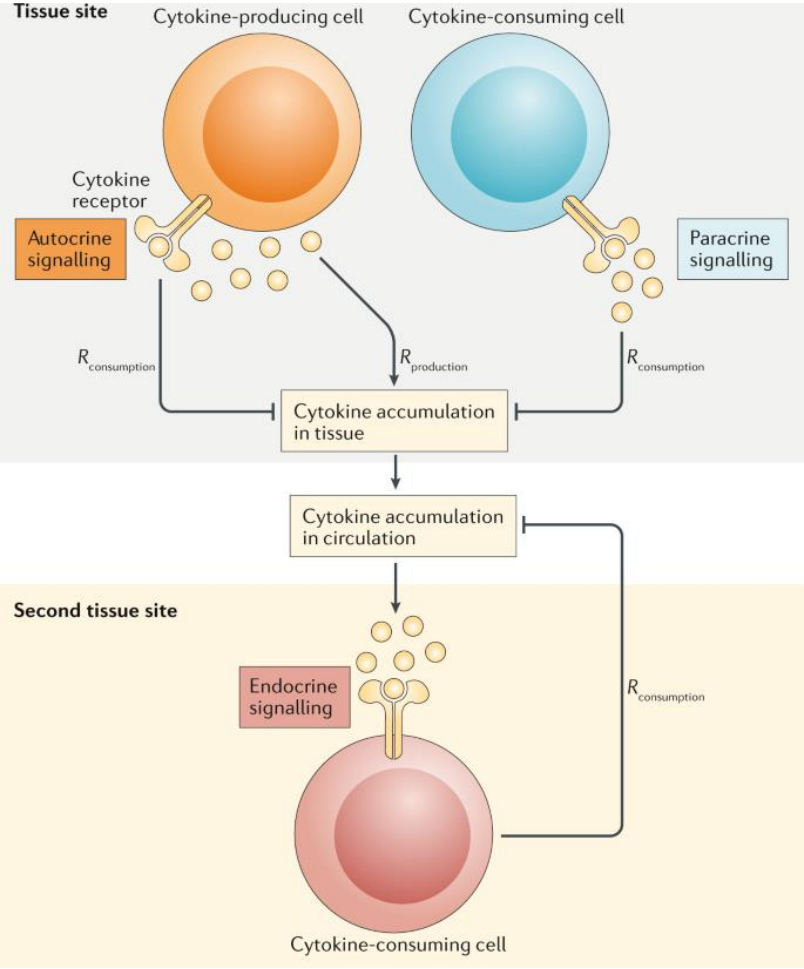
Negative proteins of acute phase

- Decreases in albumin, transferrin, cortisol-binding globulin, transthyretin and vitamin A binding protein temporarily lead to an increased supply of free hormones, which usually bind to these proteins.

- Transthyretin (pre-albumin binding thyroxine, transports thyroid hormones from the plexus choroideus to the cerebrospinal fluid) inhibits the production of IL-1β by monocytes and endothelial cells. Its decline can thus be considered as a pro-inflammatory mechanism. These changes in blood protein profiles appear to be partly related to muscle starvation and catabolism. It is also an offer of amino acids for the production of positive acute phase proteins.

Cytokines

- generic name for a diverse group of soluble proteins and peptides
- act as humoral regulators at nano- to picomolar concentrations under normal or pathological conditions
- modulate the functional activities of individual cells and tissues.
- These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment.



Nature Reviews Immunology **volume 19**, pages205–217(2019)

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Cytokine network

→This term essentially refers to the extremely complex interactions of cytokines by which they induce or suppress their own synthesis or that of other cytokines or their receptors, and antagonize or synergies with each other in many different and often redundant ways.

→These interactions often resemble Cytokine cascades with one cytokine initially triggering the expression of one or more other cytokines that, in turn, trigger the expression of further factors and create complicated feedback regulatory circuits.

→Mutually interdependent pleiotropic cytokines usually interact with a variety of cells, tissues and organs and produce various regulatory effects, both local and systemic.

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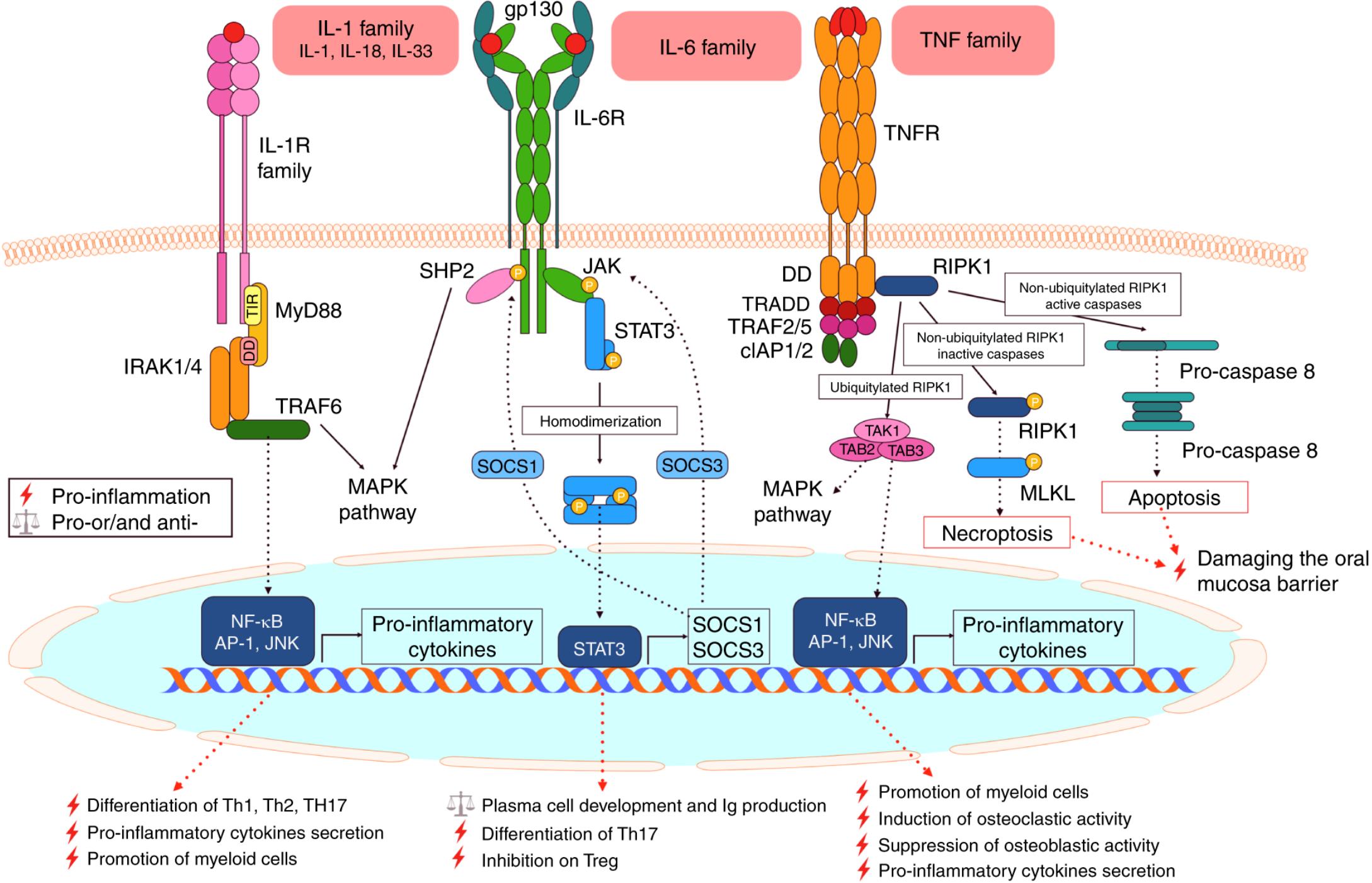
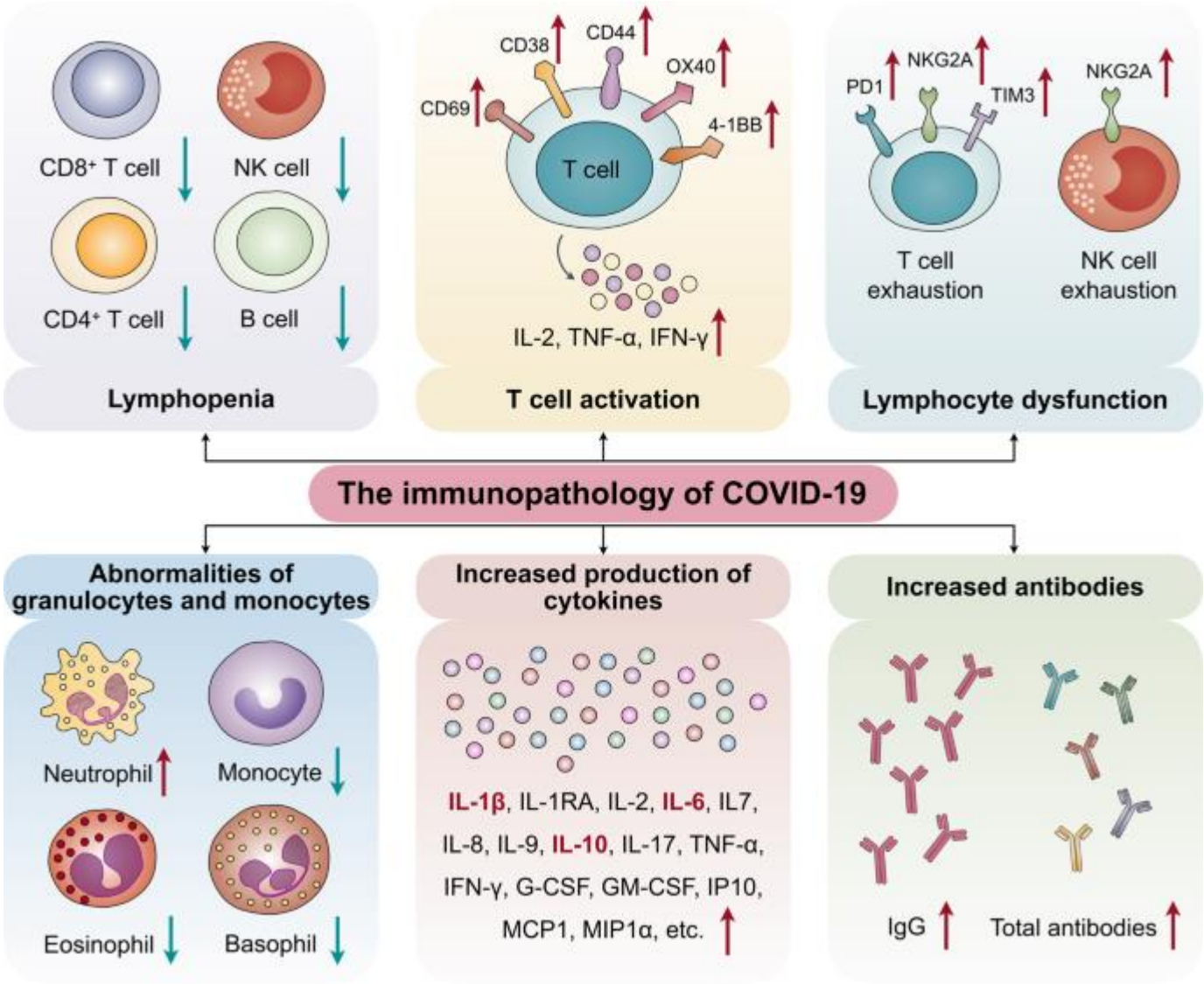

IL-1 family
IL-1, IL-18, IL-33
IL-6 family
TNF family
gp130
IL-6R
IL-1R
family
TNFR
SHP2
JAK
STAT3
DD
TRADD
TRAF2/5
cIAP1/2
RIPK1
Non-ubiquitylated RIPK1
active caspases
Non-ubiquitylated RIPK1
inactive caspases
Pro-caspase 8
TIR
MyD88
DD
IRAK1/4
TRAF6
Homodimerization
Ubiquitylated RIPK1
TAK1
TAB2
TAB3
RIPK1
Pro-caspase 8
SOCS1
SOCS3
MAPK
pathway
MAPK
pathway
MLKL
Apoptosis
Necroptosis
Pro-inflammation
Pro-or/and anti-
Damaging the oral
mucosa barrier
NF-κB
AP-1, JNK
Pro-inflammatory
cytokines
STAT3
SOCS1
SOCS3
NF-κB
AP-1, JNK
Pro-inflammatory
cytokines
Differentiation of Th1, Th2, TH17
Pro-inflammatory cytokines secretion
Promotion of myeloid cells
Plasma cell development and Ig production
Differentiation of Th17
Inhibition on Treg
Promotion of myeloid cells
Induction of osteoclastic activity
Suppression of osteoblastic activity
Pro-inflammatory cytokines secretion

“cytokine storm”

- extreme increase in inflammatory cytokines, including IL-1β, IL-2, IL-6, IL-7, IL-8, IL-10, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon-inducible protein-10 (IP10), monocyte chemotactic protein 1 (MCP1), macrophage inflammation protein-1α, IFN-γ, and TNF-α.



Signal Transduction and Targeted Therapy **volume 5**, Article number: 128 (2020)

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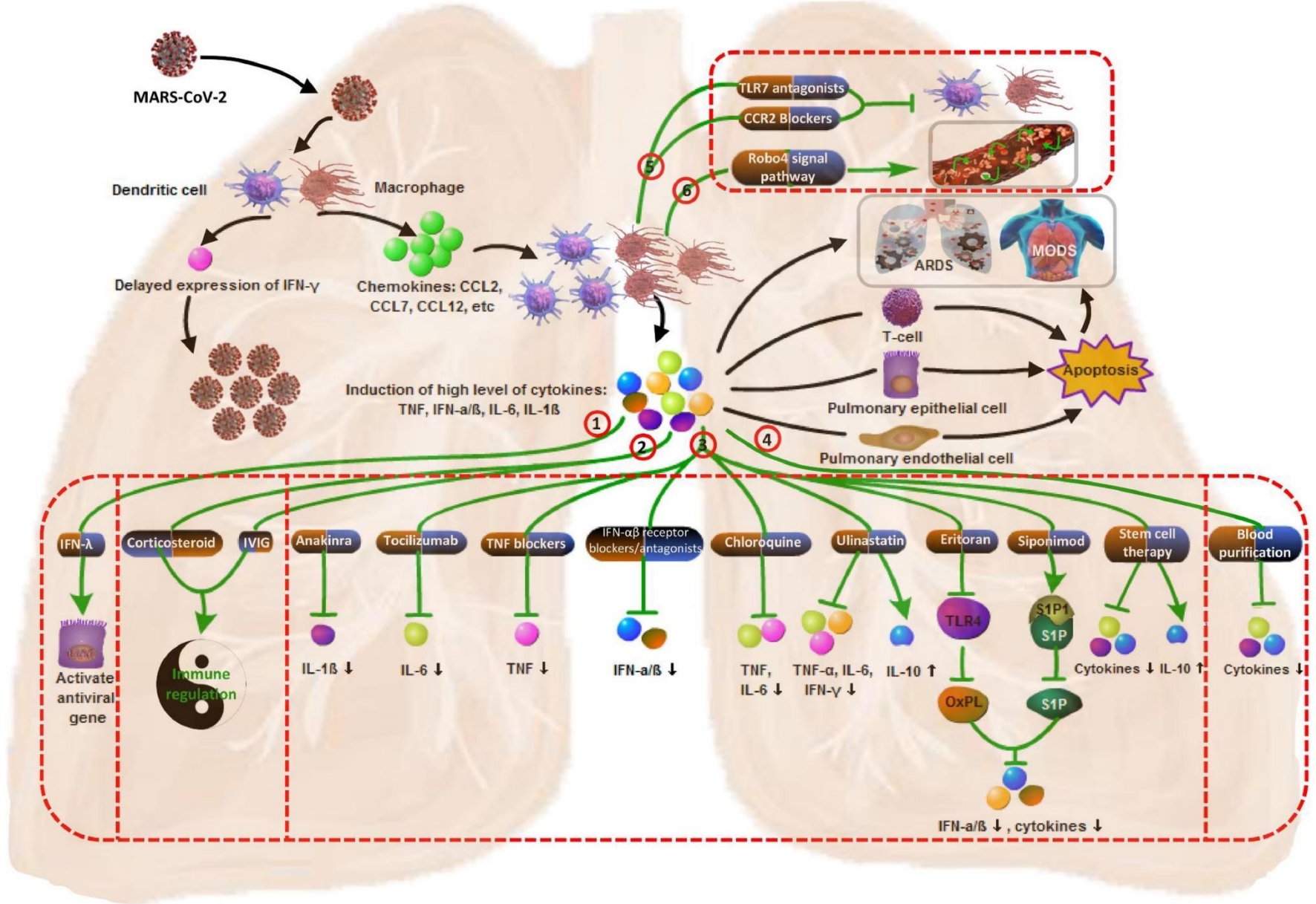

Journal of Infection 80 (2020) 607-613

Chemokines

- ✓Generic name given to a family of pro-inflammatory activation-inducible cytokines. These proteins are mainly chemotactic for different cell types.
- ✓All chemokines possess a number of conserved cysteine residues involved in intramolecular disulfide bond formation, which allows chemokines to be grouped into families according to the presence or absence of one or more conserved cysteine residues.

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Chemokines

- According to their mode of expression and function, chemokines have been categorized as inflammatory chemokines and homeostatic chemokines.
  - Inflammatory chemokines are expressed usually by leukocytes or related cells only upon cell activation. These factors mediate emigration of leukocytes.
  - Homeostatic chemokines are expressed constitutively and are involved usually in relocation of lymphocytes or other cell types.
  - Dual-function chemokines can act as inflammatory cytokines or homeostatic cytokines.

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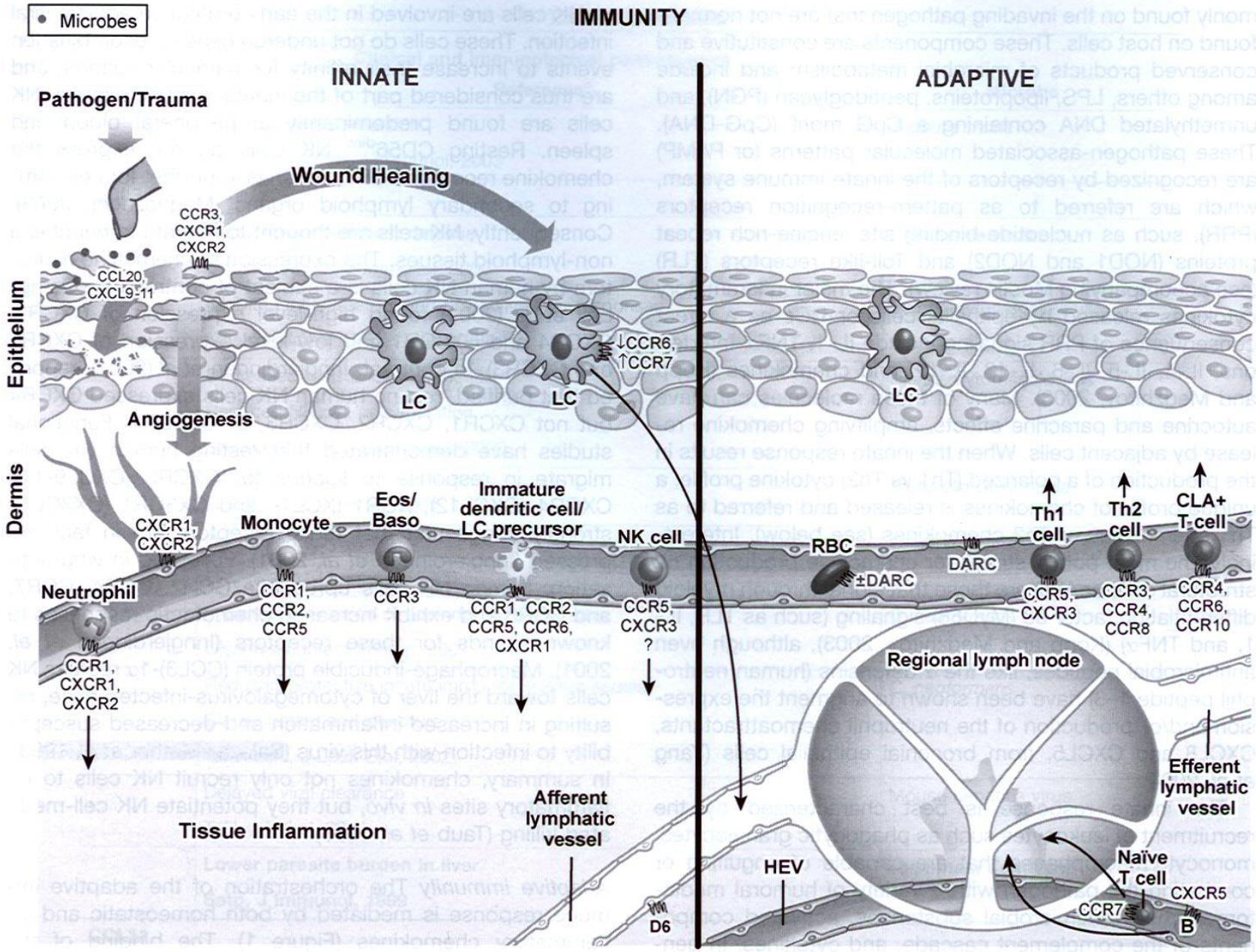
Microbes
IMMUNITY
INNATE
ADAPTIVE
Pathogen/Trauma
Wound Healing
CCR3, CXCR1, CXCR2
CCL20, CXCL9-11
Epithelium
Dermis
LC
↓CCR6, ↑CCR7
Angiogenesis
CXCR1, CXCR2
Neutrophil
CCR1, CXCR1, CXCR2
Monocyte
CCR1, CCR2, CCR5
Eos/ Baso
CCR3
Immature dendritic cell/ LC precursor
CCR1, CCR2, CCR5, CCR6, CXCR1
NK cell
CCR5, CXCR3 ?
RBC
±DARC
DARC
Th1 cell
CCR5, CXCR3
Th2 cell
CCR3, CCR4, CCR8
CLA+ T cell
CCR4, CCR6, CCR10
Regional lymph node
Efferent lymphatic vessel
Afferent lymphatic vessel
Tissue Inflammation
HEV
D6
Naïve T cell
CCR7
CXCR5
B

Wound healing

Wound healing

- Wound healing is the process of repair that follows injury to the skin and other soft tissues.
- Healing is the interaction of a complex cascade of cellular events that generates resurfacing, reconstitution, and restoration of the tensile strength of injured tissue.
- Under the most ideal circumstances, healing is a systematic process, traditionally explained in terms of 3 classic phases: inflammation, proliferation, and maturation.

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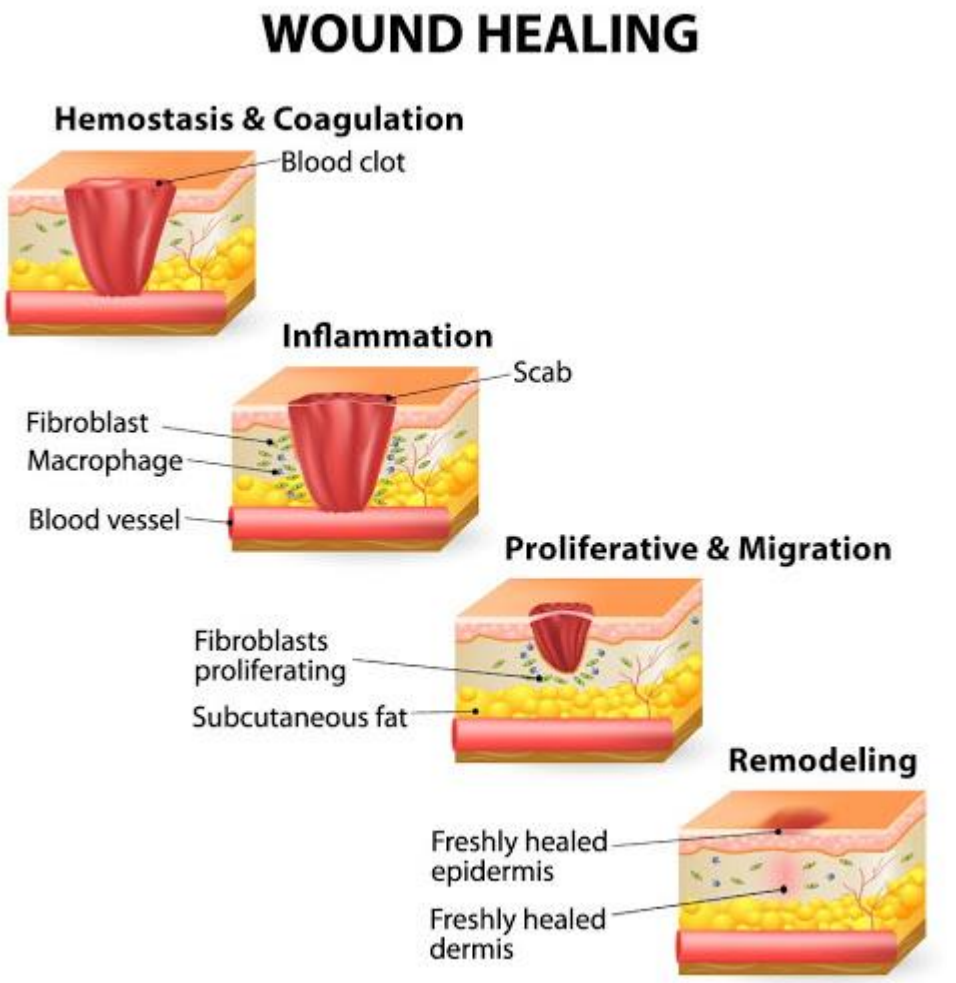
Wound healing

- **The inflammatory phase**:
  - a clot forms and cells of inflammation debride injured tissue.
- **The proliferative phase**:
  - epithelialization, fibroplasia, and angiogenesis occur; additionally, granulation tissue forms and the wound begins to contract.
- **The maturation phase**:
  - Collagen forms tight cross-links to other collagen and with protein molecules, increasing the tensile strength of the scar.



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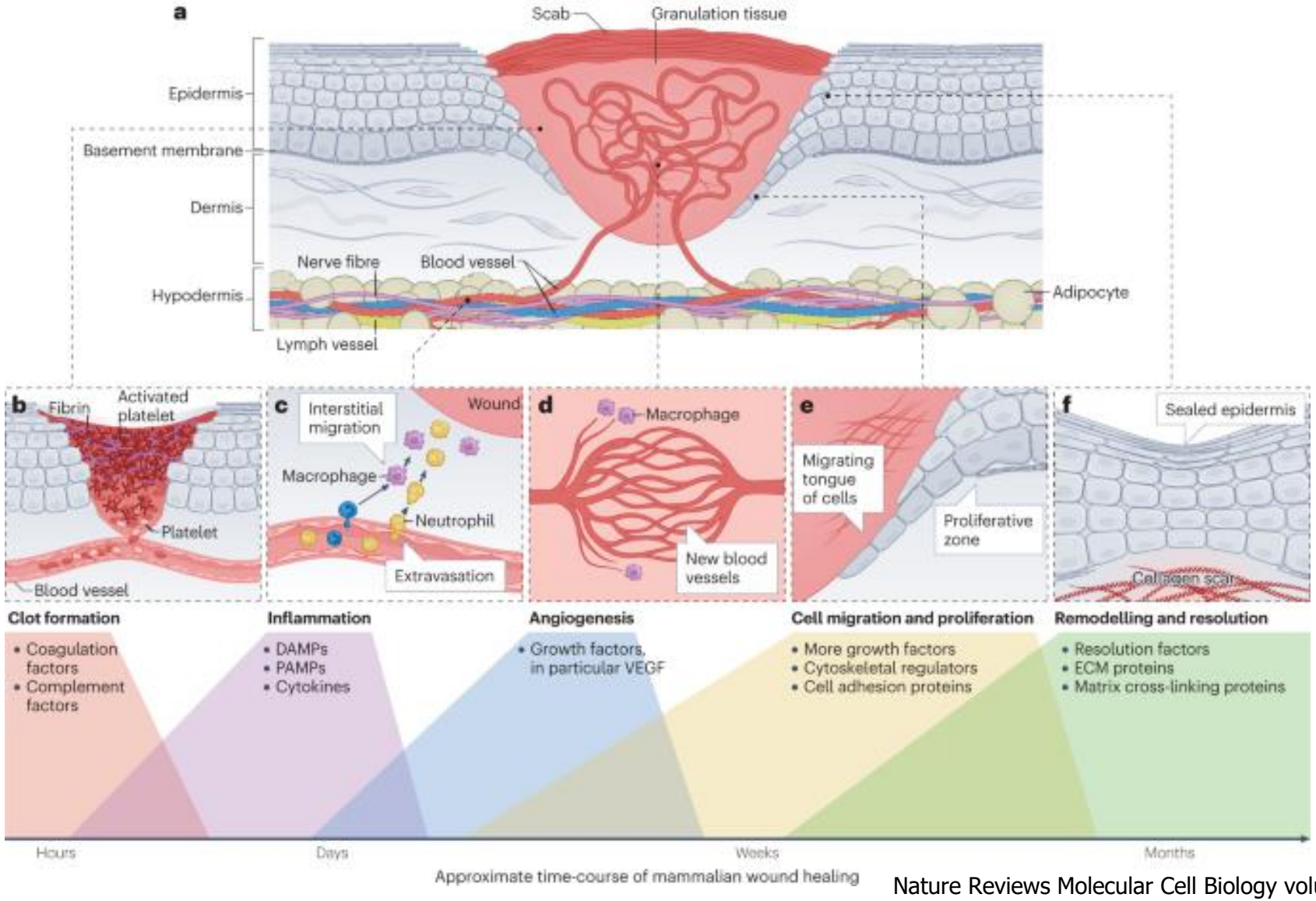
Phases of wound healing



Nature Reviews Molecular Cell Biology volume 25, pages599–616 (2024)

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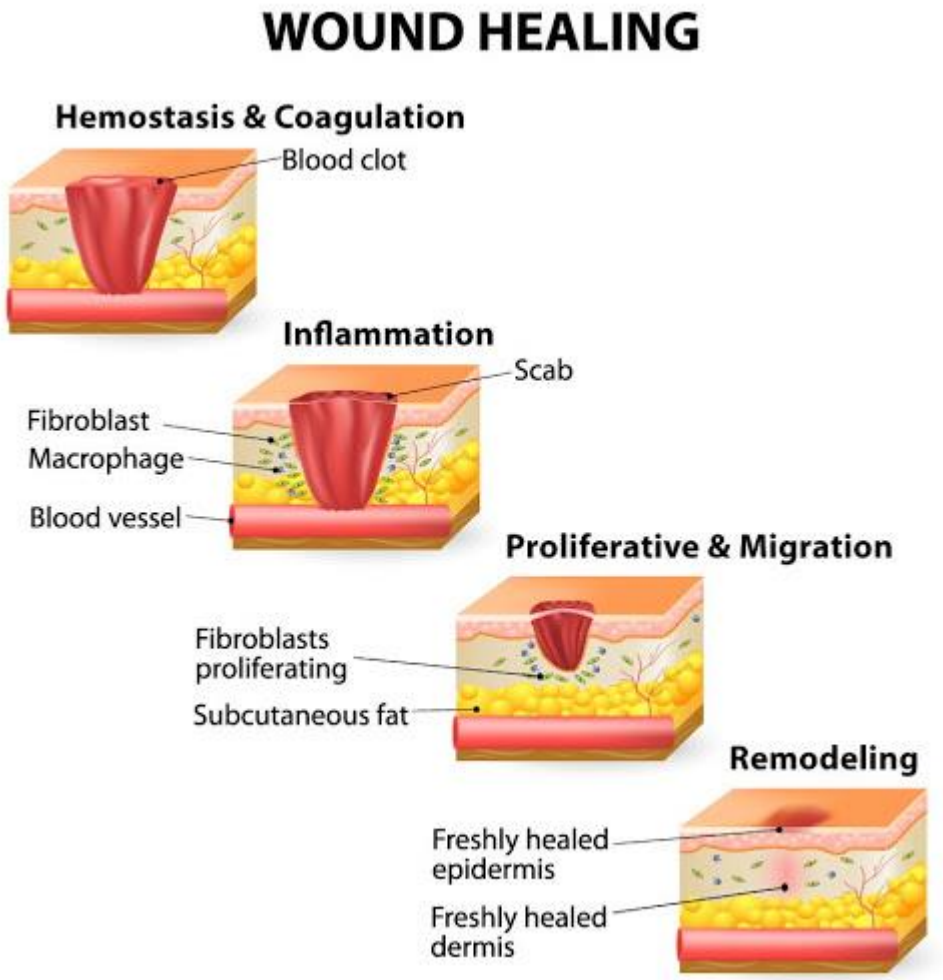
I. Inflammatory Phase

Immediate to 2-5 days

- **Hemostasis**
  - Vasoconstriction
  - Platelet aggregation
  - Clot formation
- **Inflammation**
  - Vasodilation
  - Phagocytosis
- Fibrin products
  - essential to wound healing and
  - primary component of the wound matrix into which inflammatory cells, platelets, and plasma proteins migrate.
  - Removal of the fibrin matrix impedes wound healing.



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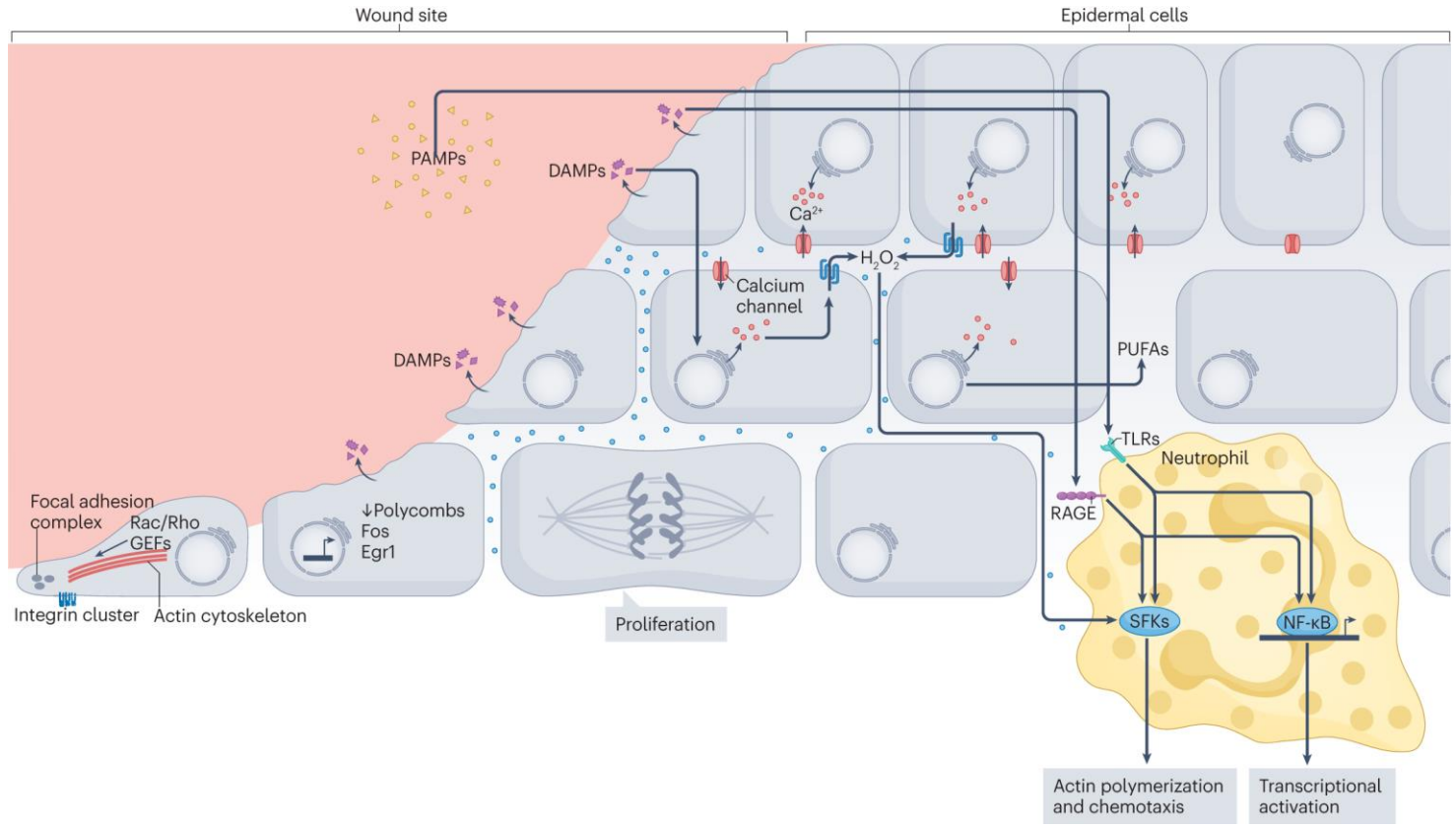
Activation of inflammation

- damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) from damaged neighbouring cells and microorganisms, and both will activate various Toll-like receptors (TLRs) and receptors such as receptor for advanced glycosylation end products (RAGE)
- DAMPs or any membrane damage to wound edge cells will trigger Ca^{2+} influx into the cytoplasmic space
- Ca^{2+} wave and the release of ROS generated by Nox (NADPH oxidase) and Duox (dual oxidase) enzymes



Nature Reviews Molecular Cell Biology volume 25, pages599–616 (2024)

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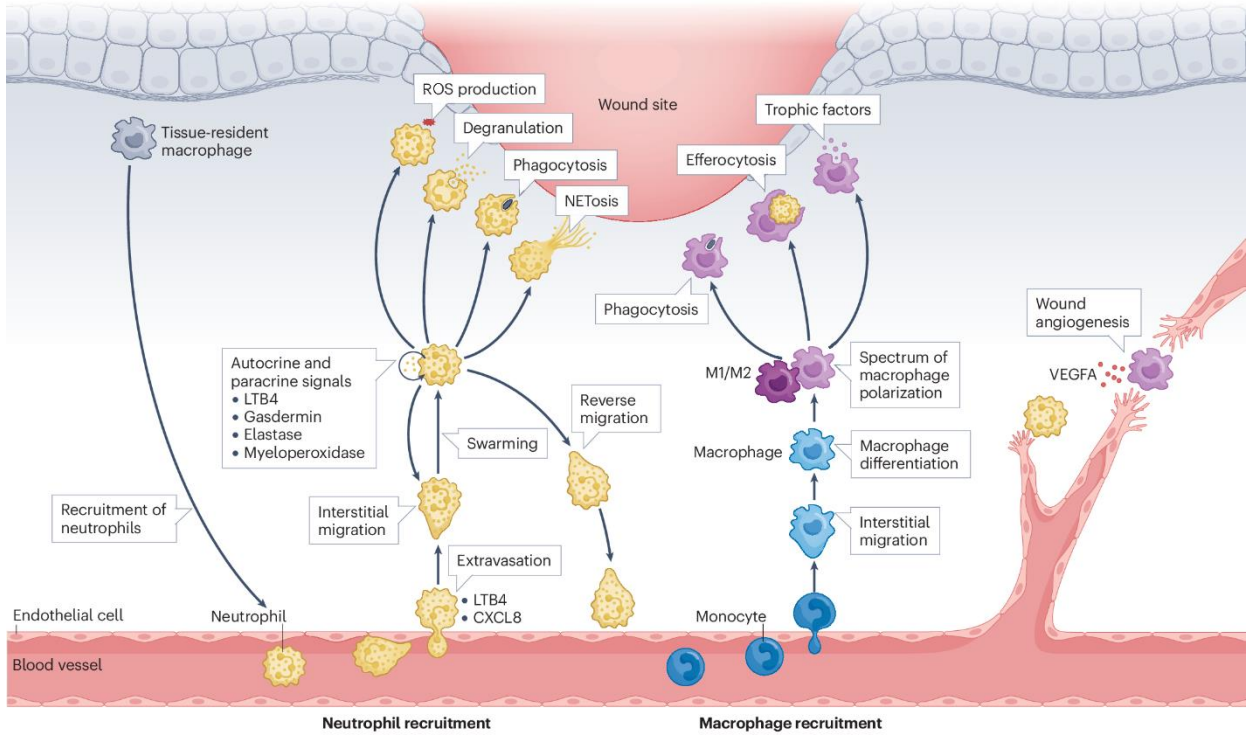
Wound inflammatory response and the roles of neutrophils and macrophages

- Neutrophils are recruited
  - passively through blood loss or by active extravasation from the vasculature.
- macrophages
  - local tissue residents or derive from extravasating monocytes from the vasculature.
- Extravasated immune cells migrate interstitially towards the wound following gradients of different inflammatory signals such as H_2O_2, DAMPs and PAMPs.
  - at the site of the wound - release signals to recruit more inflammatory cells



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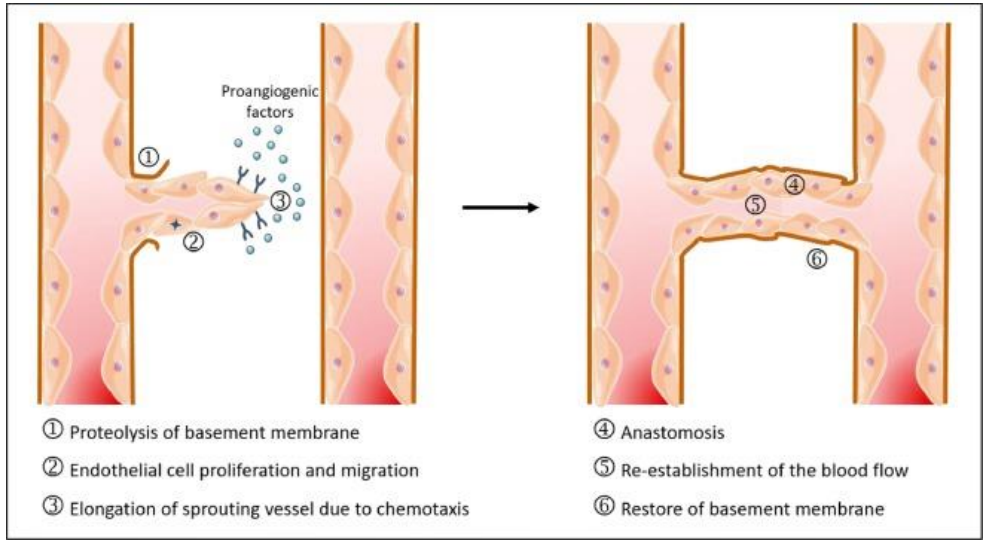
II. Proliferative Phase

2 days to 3 weeks

- **B) Granulation**
  - Fibroblasts lay bed of collagen - scaffold for migration and further fibroblast proliferation
- **C) Contraction**
  - Wound edges pull together to reduce defect
- **D) Epithelialization**
  - Crosses moist surface
  - Cell travel about 3 cm from point of origin in all directions



Journal of Theoretical Biology 459, 2018, 1-17

vascular network is also re-established through **angiogenesis**

- main regulator of angiogenesis is the vascular endothelial growth factor (VEGF) family, which includes VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF)

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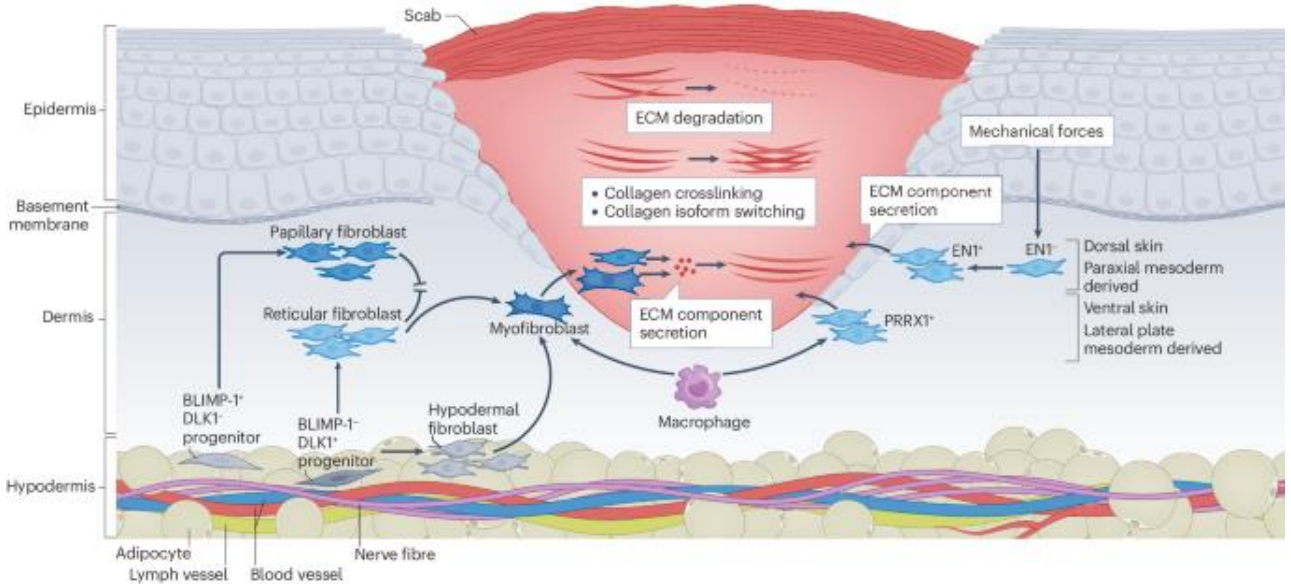
Role of fibroblasts

- Various fibroblastic lineages from different anatomical locations contribute to the deposition of wound matrix mediated by mechanical signals and signals from inflammatory macrophages
  - microanatomical cellular differences in contribution to scarring,
  - gross anatomical locations of the body have different predispositions to scarring, with some sites being very prone to scaring, for example, the back, whereas other sites, most notably the mouth, tend to be almost scar resistant



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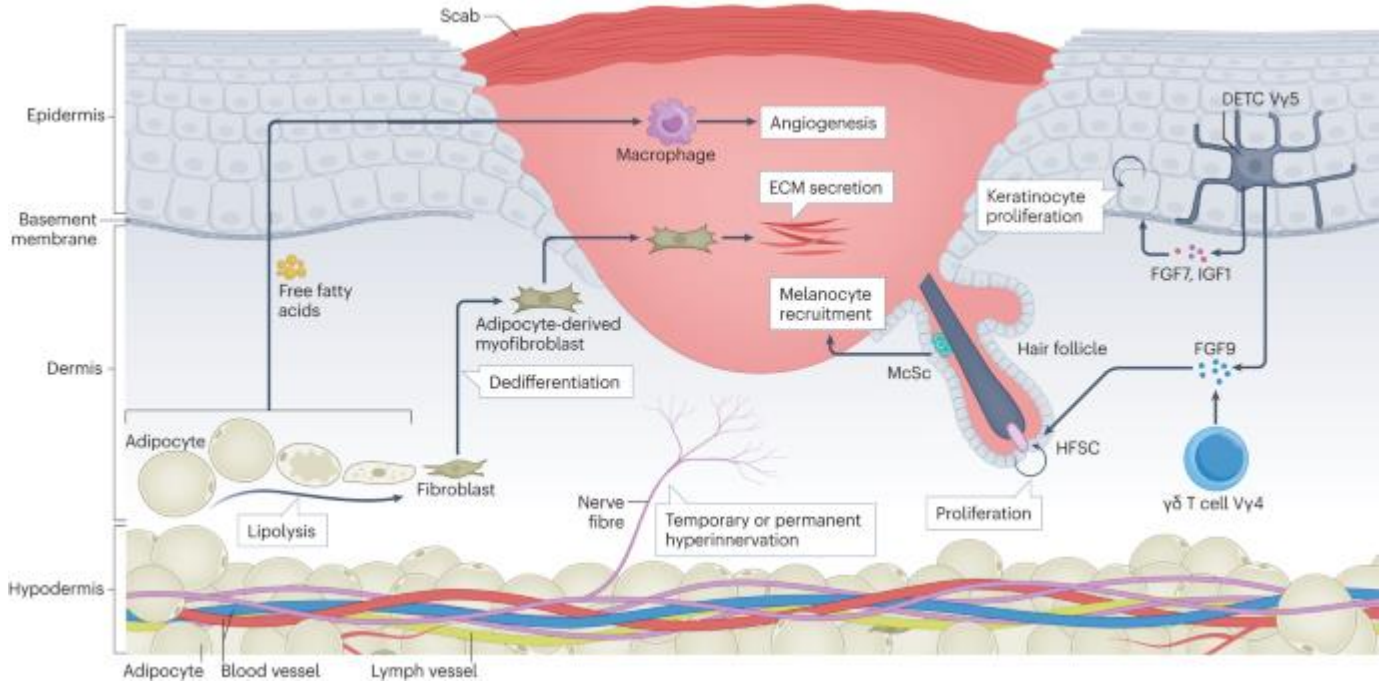
Roles of other cell types in wound healing

- adipocytes at the wound edge
  - undergo lipolysis and release free fatty acids that activate macrophages and induce angiogenesis
  - dedifferentiate to become myofibroblasts and then migrate into the wound bed and secrete extracellular matrix (ECM) components
- melanoblasts and melanocytes
- mast cells
- neurones



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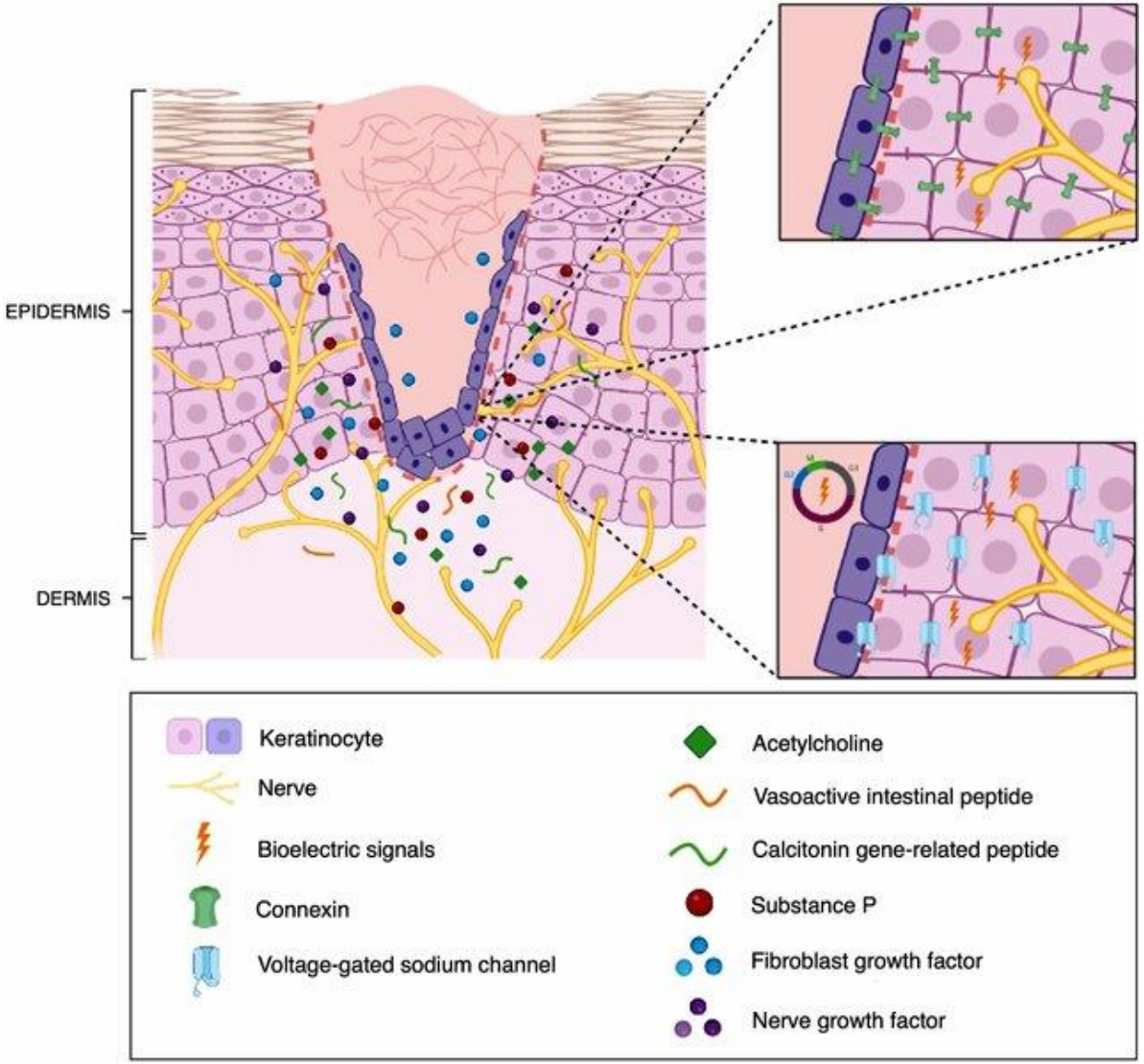
Nerves are important

- Nerves fibers infiltrate the epidermis and dermis of the skin. After injury, nerves secrete growth factors, neurotransmitters and neuropeptides, modulate gap junction expression, and influence bioelectric signaling to drive keratinocyte proliferation and migration during reepithelialization and wound healing.



August 2023, Developmental Dynamics 253(2)

MUNI MED

III. Maturation Phase

- During the maturation phase, fibroblasts leave the wound and collagen is remodeled into a more organized matrix.
- Tensile strength increases for up to one year following the injury. While healed wounds never regain the full strength of uninjured skin, they can regain up to 70 to 80% of its original strength.

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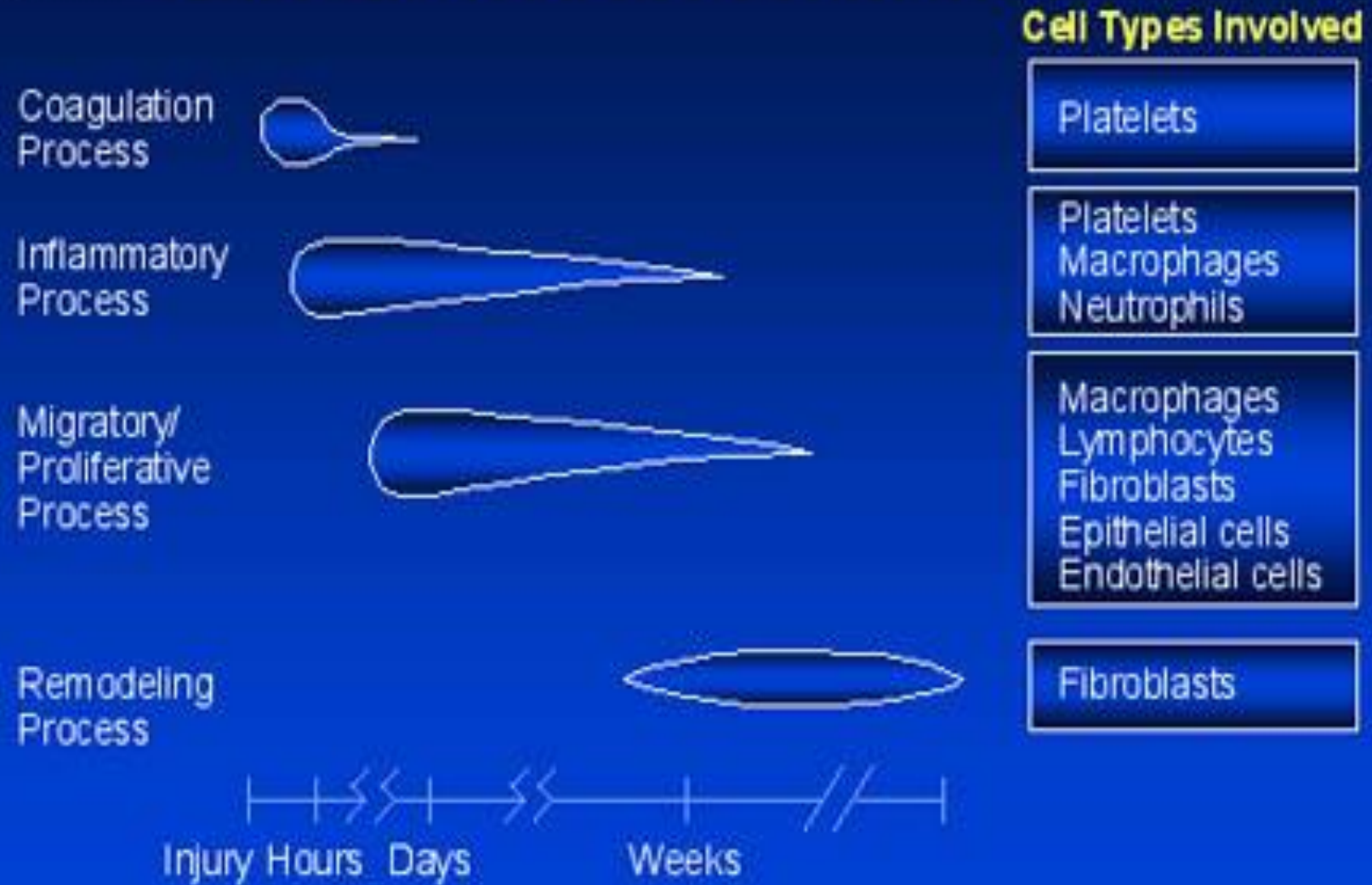
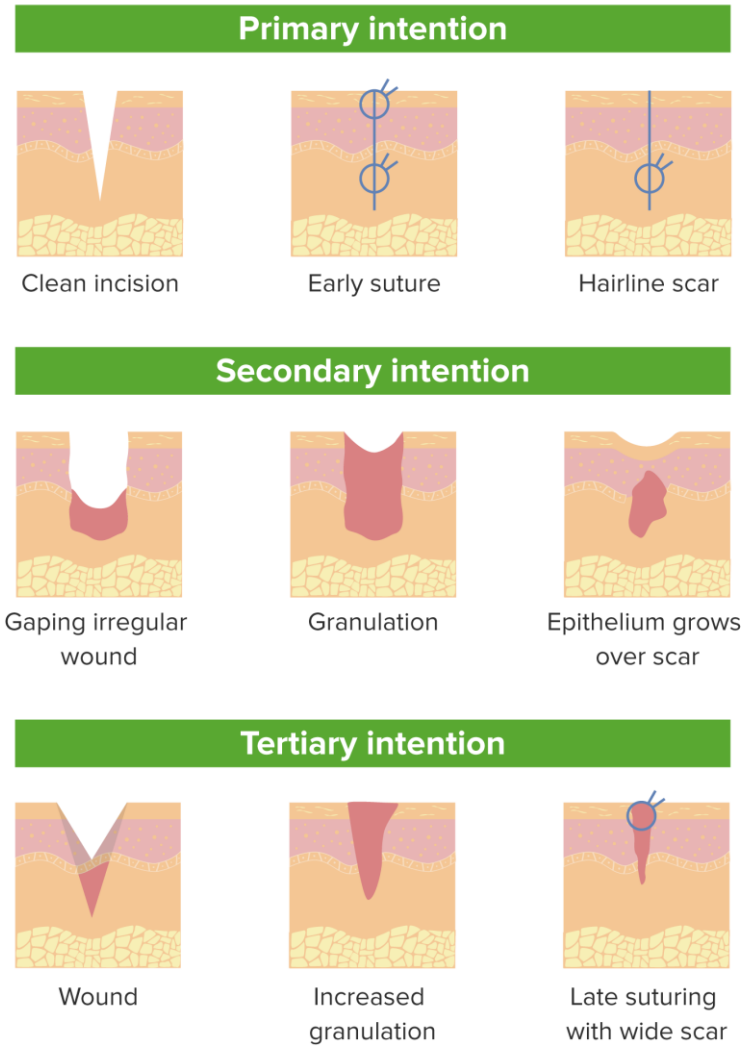
COMPONENTS OF WOUND HEALING



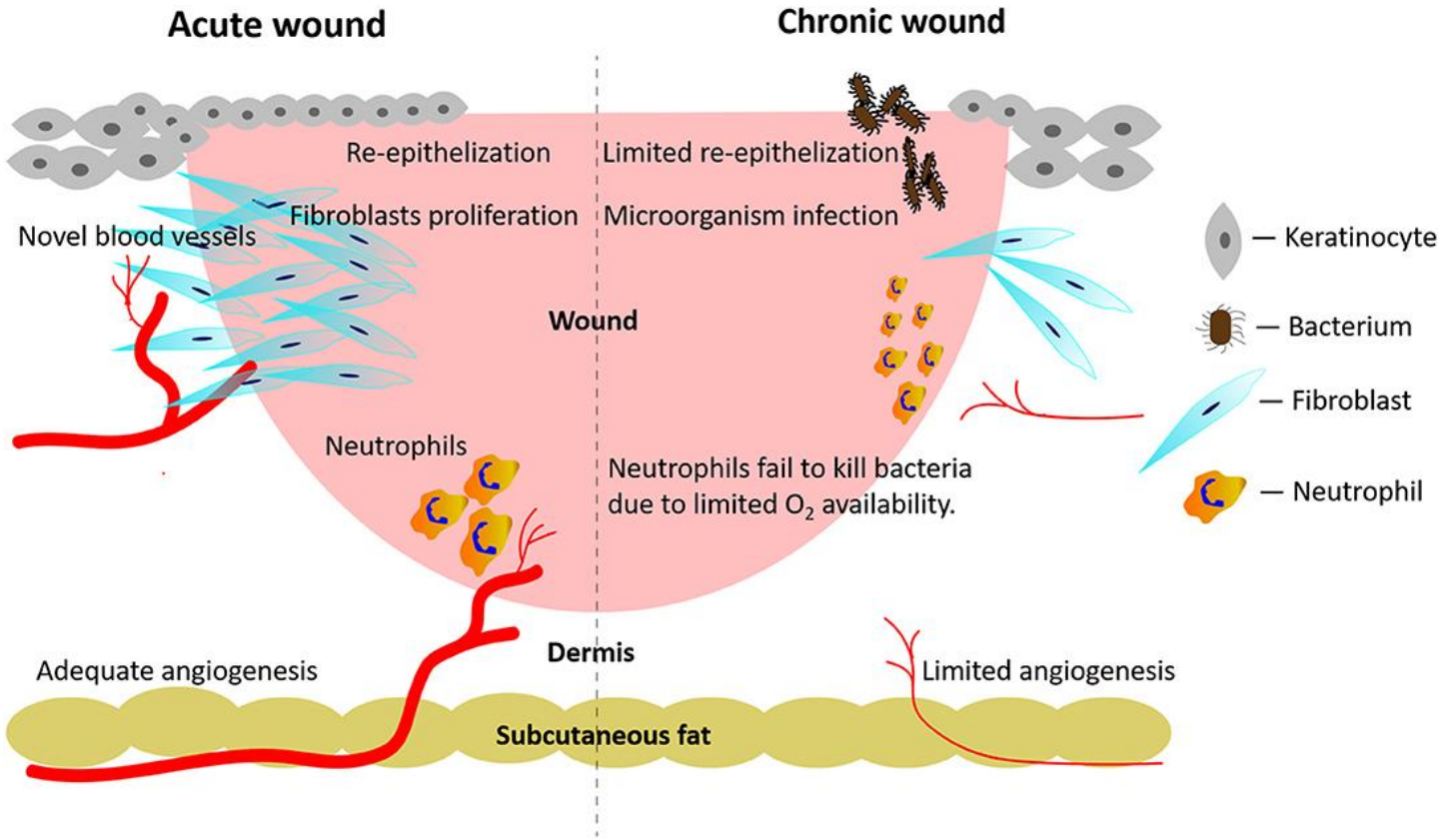
Kane DP, Krasner D. In: *Chronic Wound Care*. 2nd ed. Health Management Publications Inc; 1997:1-4.

First intention healing occurs when there is no tissue loss, and the edges of the skin or its components are closely juxtaposed, resulting in relatively rapid healing.
Second intention healing, conversely, is observed when tissue loss is significant, and the edges of the skin are distant, leading to a slower healing process.
Third intention healing involves surgically correcting wounds after the formation of granulation tissue or to control infection, ultimately yielding improved functional and aesthetic outcomes.



Acute vs. chronic wound

- Acute wounds
  - adequate angiogenesis promotes re-epithelialization, fibroblasts' proliferation, and neutrophils' anti-infection activities.
- Chronic wounds
  - persistent local bacterial infections hinder the formation of novel blood vessels. The restricted angiogenesis hampers fibroblasts' proliferation and the neutrophils' anti-infection activities.



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Factors affecting wound healing

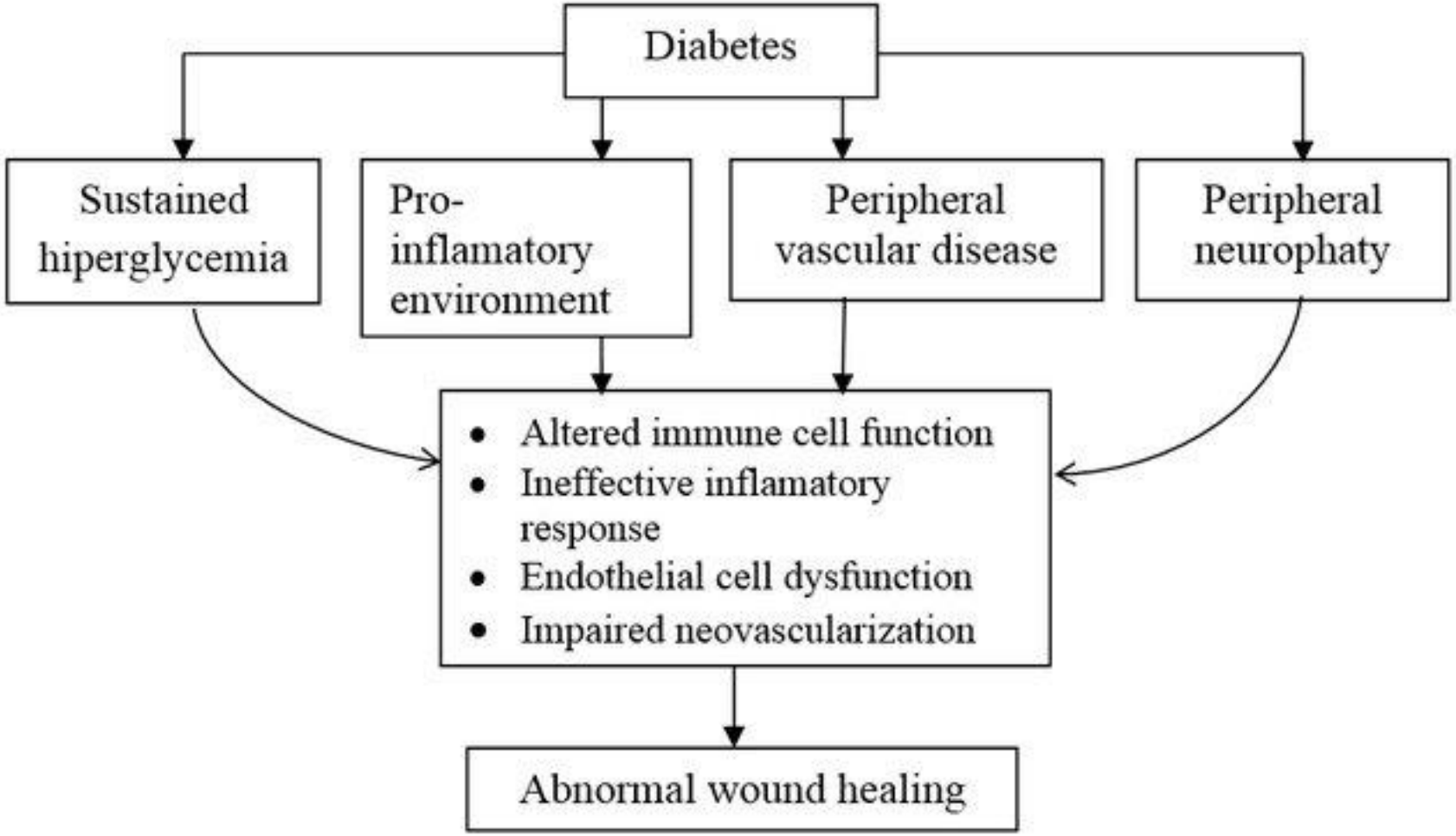
Systemic factors

- Age
- Nutrition
- Trauma
- Metabolic diseases
- Immunosuppression
- Connective tissue disorders
- smoking

Local factors

- Mechanical injury
- Infection
- Edema
- Topical agents
- Ionizing radiation
- Necrotic tissue
- Low oxygen tension
- Foreign bodies

Wound healing in DM



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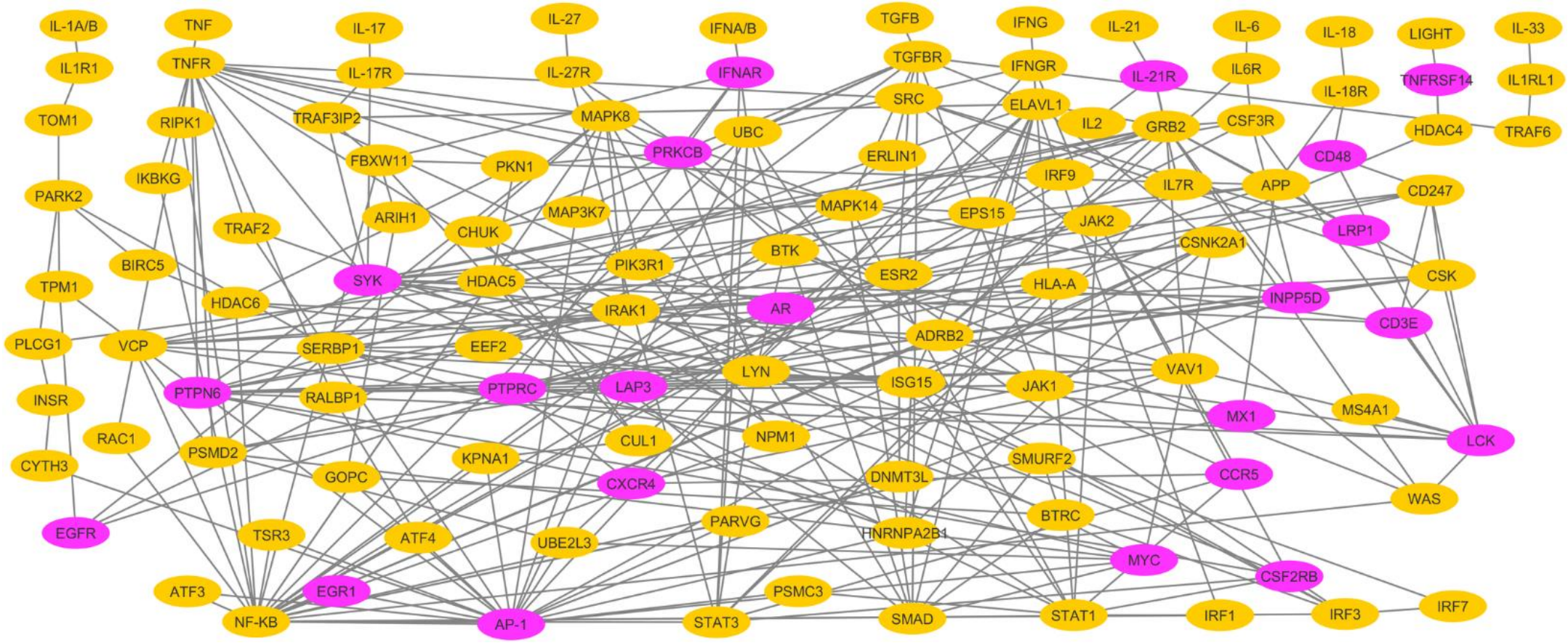
Thank you for attention



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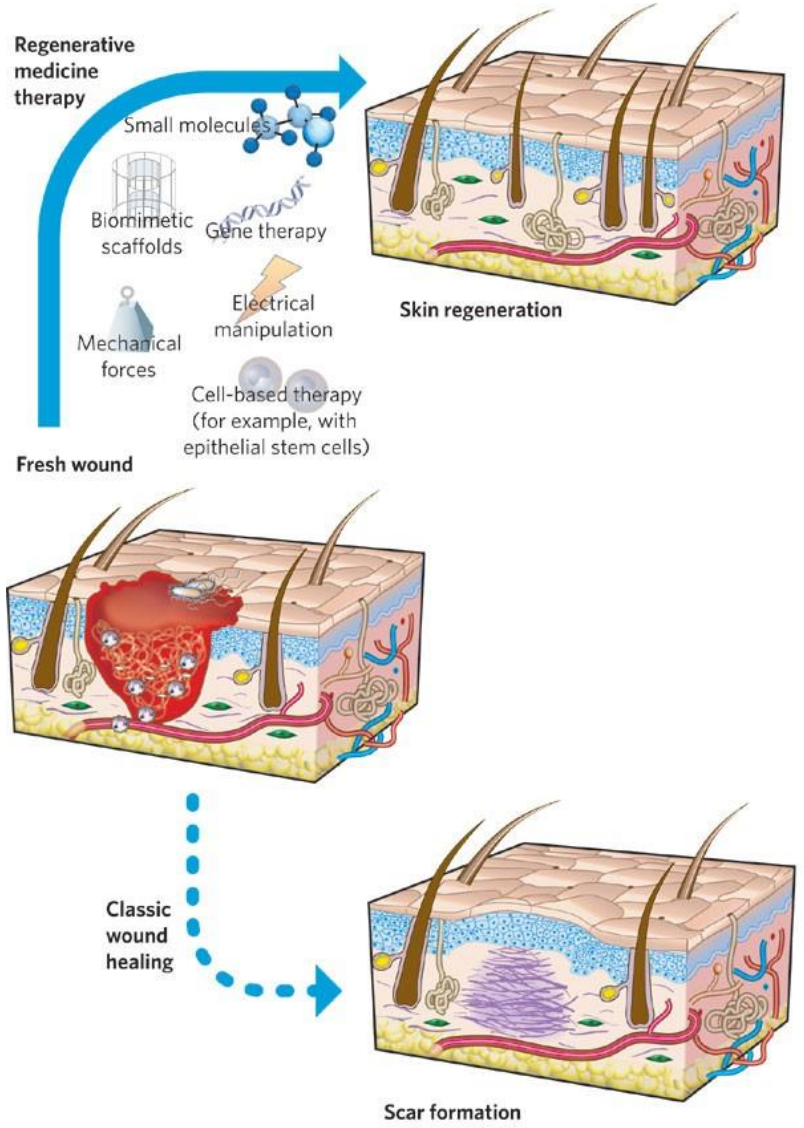
Regenerative medicine



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