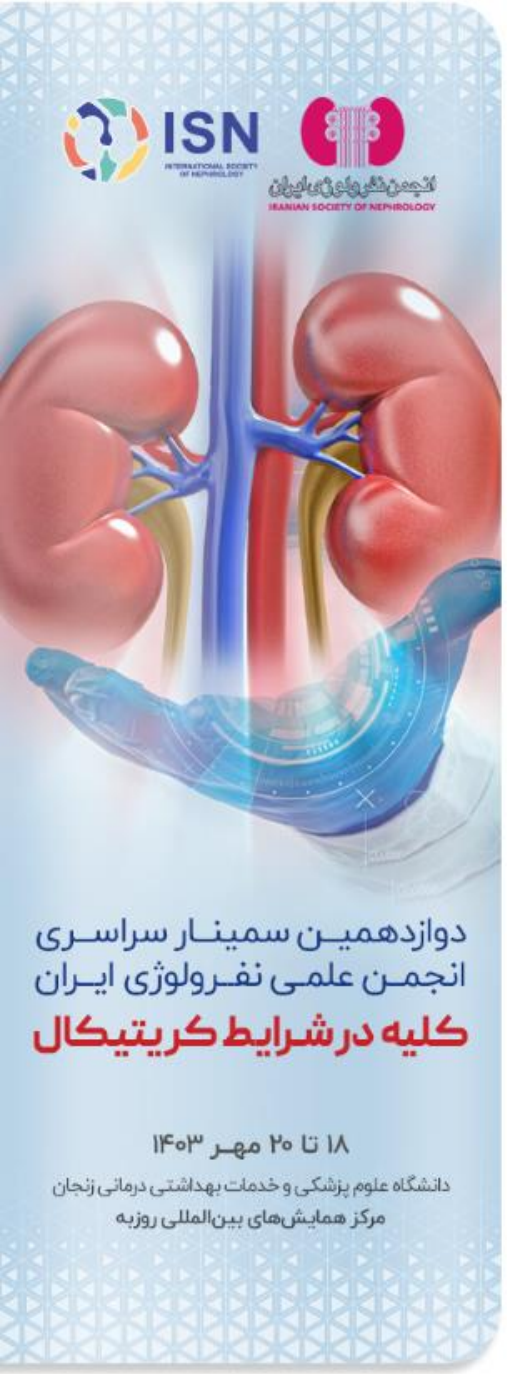

The Role of Oxidative Stress and Inflammation in AKI

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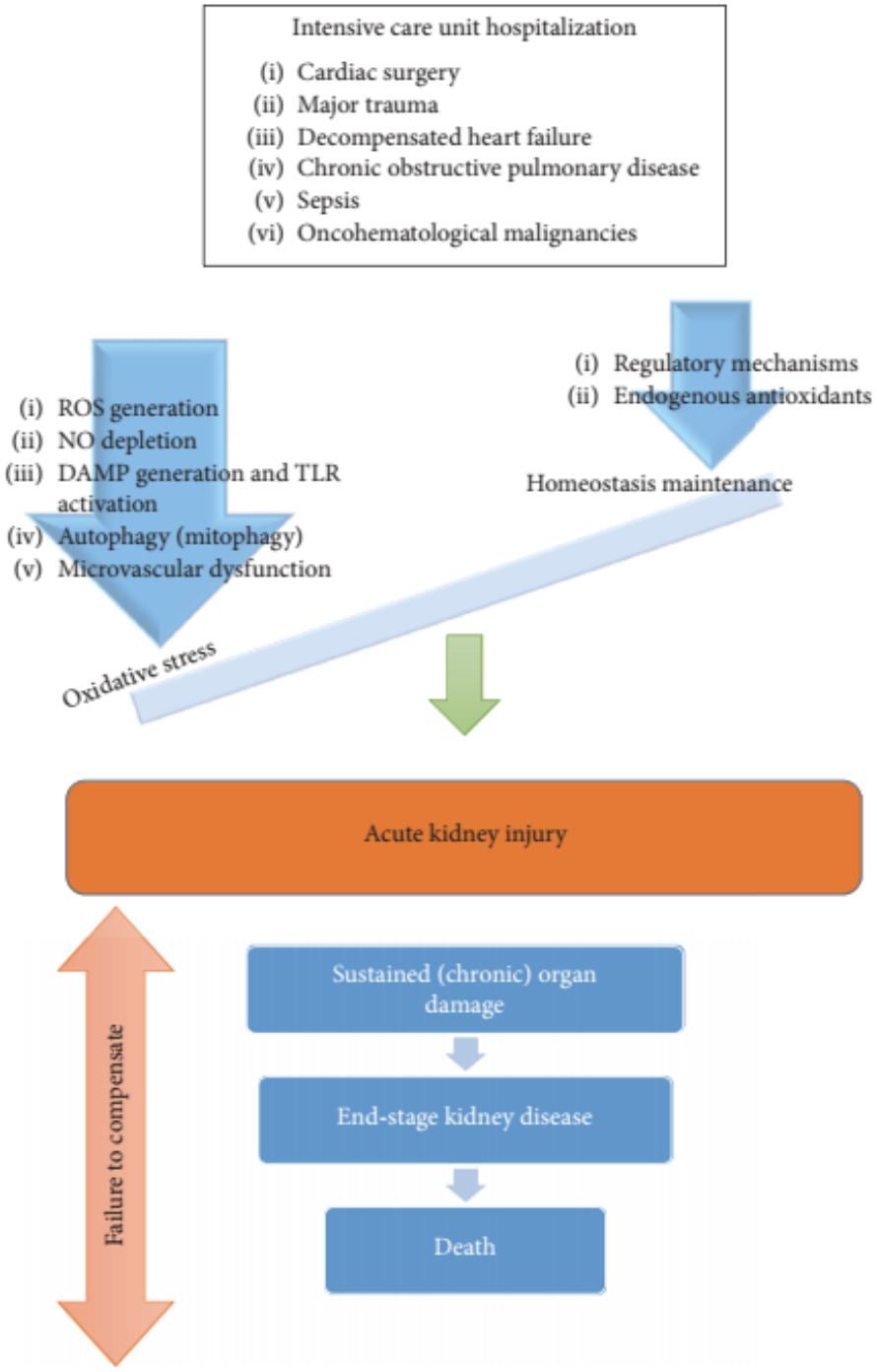
✓*Acute Kidney Injury (AKI)* a major public health problem

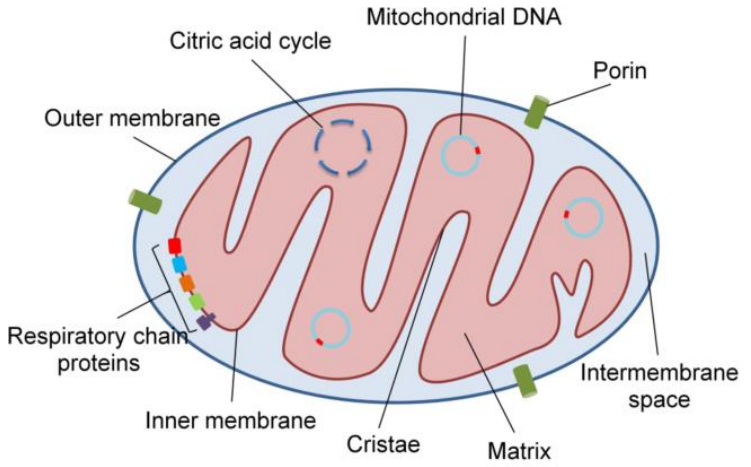
- ✓ Reported incidence of 0.25% in general population and 18% in hospitalized patients
- ✓ All-cause AKI incidence in ICU patients up to 36%
- ✓ An independent risk factor for **patients' outcome and mortality**
- ✓ Prolongation of hospitalization
- ✓ Occasional progress to **chronic kidney disease**
- ✓ *Sepsis* is the leading cause of AKI in severely ill patients in ICU (**nearly 50% of cases**)

Oxidative Stress and Its Pathogenetic Role in AKI.

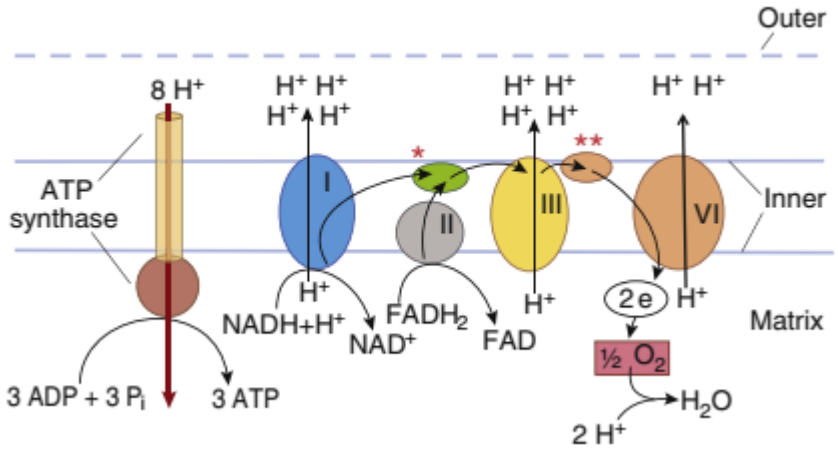
- ✓ Oxidative metabolism is for energy production
- ✓ ***Oxidative stress*** was introduced for the first time **by Stahland and Sies in 1985**:
  - ✓ Increased production of oxidants (a chain-like response, production of ROS and metabolic products)
  - ✓ Depletion of endogenous antioxidants
  - ✓ Cellular damage, **dysfunction of proteins, and damage of DNA, lipids, and enzymes**
  - ✓ Ligands for toll like receptors (TLRs)
  - ✓ It's activation is the "***alarm***" for an ongoing harmful process in AKI
- ✓ When organisms sense a possible threat:
  - ✓ ***delay metabolic processes***
  - ✓ ***cell cycle arrest*** (avoid further oxidative damage)


Intensive care unit hospitalization
(i) Cardiac surgery
(ii) Major trauma
(iii) Decompensated heart failure
(iv) Chronic obstructive pulmonary disease
(v) Sepsis
(vi) Oncohematological malignancies
(i) ROS generation
(ii) NO depletion
(iii) DAMP generation and TLR activation
(iv) Autophagy (mitophagy)
(v) Microvascular dysfunction
(i) Regulatory mechanisms
(ii) Endogenous antioxidants
Homeostasis maintenance
Oxidative stress
Acute kidney injury
Failure to compensate
Sustained (chronic) organ damage
End-stage kidney disease
Death

Reactive Oxygen Species (ROS) and Nitric Oxide (NO)



- ✓ *Mitochondrion:*
  - ✓ Primary energy factory
  - ✓ Abundant in proximal renal tubule
  - ✓ Renal cortex a crucial field of oxygen use for energy production
  - ✓ In AKI, mitochondrial injury **precedes the increase of serum creatinine levels**
- ✓ Main source of ROS generation is **reduction of oxygen by cytochrome oxidase** in mitochondrial electron chain transport (**ETC**) that results in production of:
  - ✓ Hydrogen peroxide (H2O2)
  - ✓ Superoxide anion radical (O2 -)
  - ✓ Hydroxyl radical (HO-)



✓ ***ROS*** attack on **lipids, proteins, and amino acids**:

- ✓ Results in formation of unstable molecules that act as radicals
- ✓ Finally convert into compounds with multiple metabolic effects
- ✓ **Lipid, protein, and nucleic acid peroxides** belong in ROS family

✓ *Kidney* receives about **25% of total blood supply** and is **rich in mitochondria** that render it susceptible to damage from ROS and subsequent development of AKI:

- ✓ *Cellular apoptosis*
- ✓ *Lipid, protein, nucleic acid peroxidation*
- ✓ *Imbalanced calcium concentration*

eNOS Main source of NO production from arginine and oxygen:

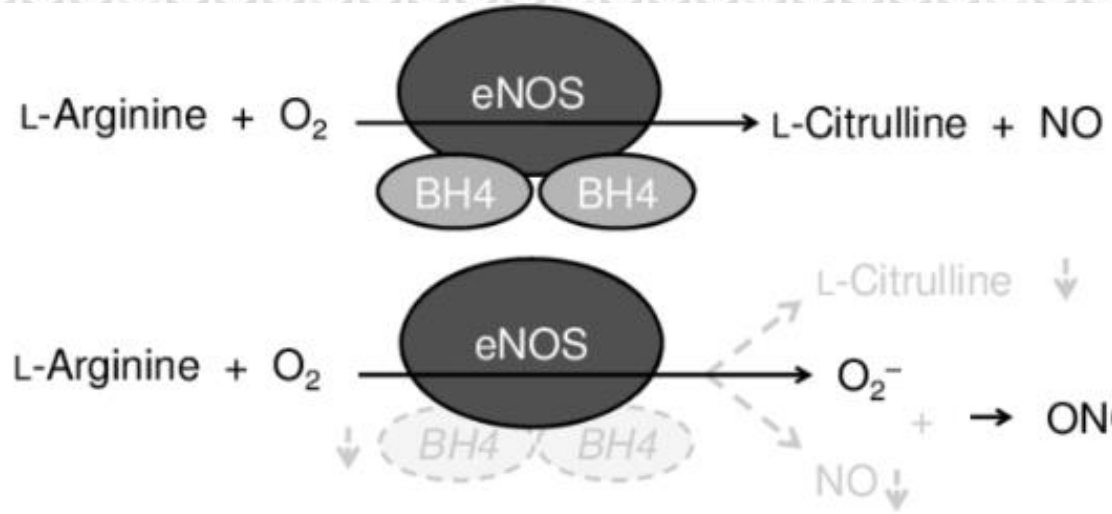
- ✓ Essential for normal endothelial function and vascular tone
- ✓ Prevention of platelet aggregation
- ✓ Presenting anti-inflammatory properties

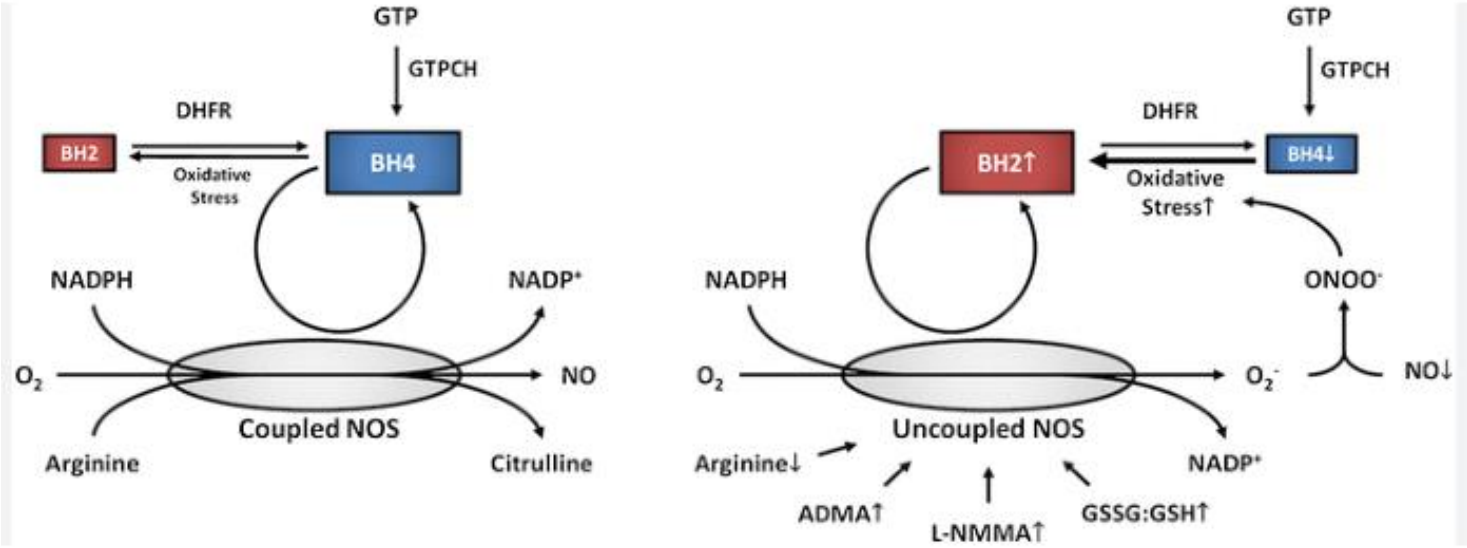
"Uncoupling" phenomenon is met when:

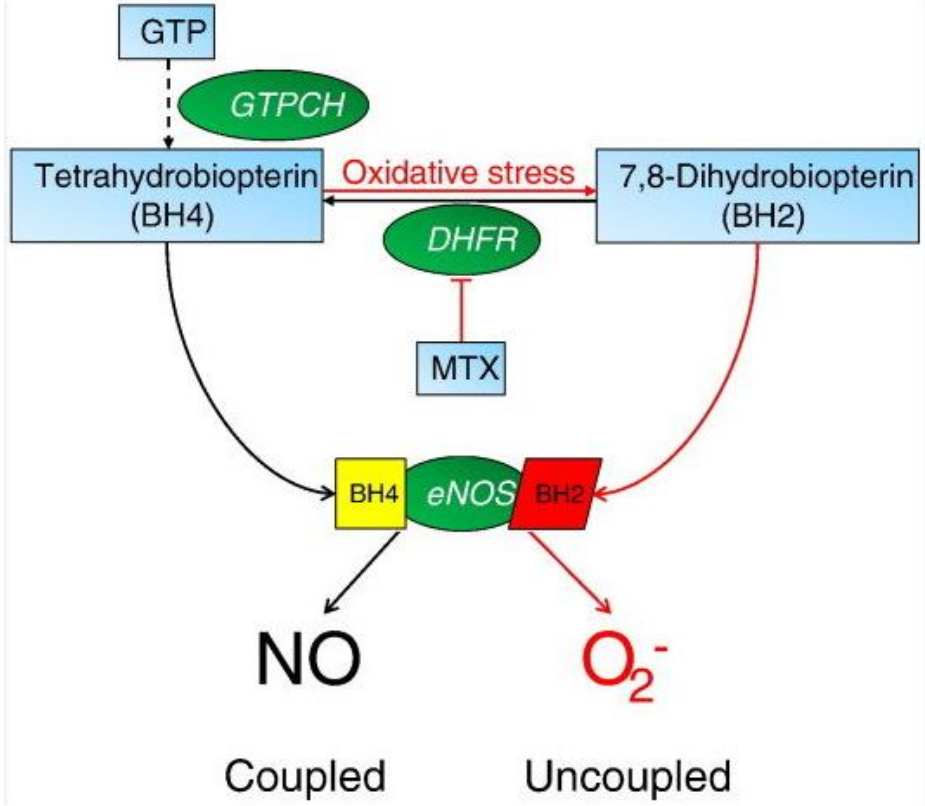
- ✓ *eNOS* is deprived of its cofactors (**calmodulin and tetrahydrobiopterin**) in inflammatory situations and sepsis
- ✓ Results in **oxidation of oxygen and release of superoxide** (and *oxygen consumption*)
- ✓ Acts as a free radical adding on to oxidative stress
- ✓ An incremental cellular NO release mediated by action of iNOS

*iNOS-*dependent inhibition of eNOS:

- ✓ Deteriorates endothelial function
- ✓ A triangle among *ROS, NO, and oxygen* in pathophysiology of AKI and oxidative stress

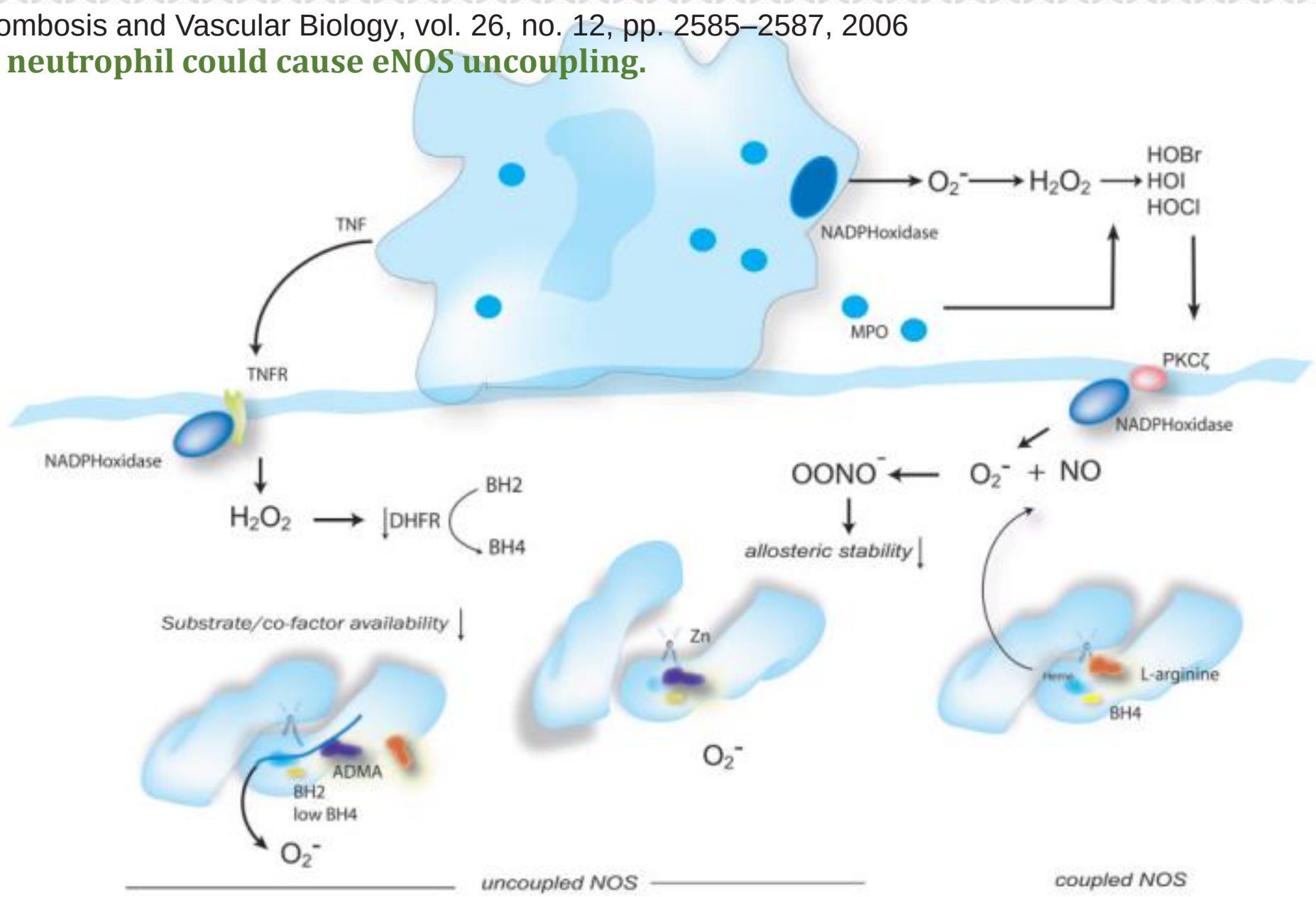

L-Arginine + O_2
eNOS
BH4
BH4
L-Citrulline + NO
L-Arginine + O_2
eNOS
BH4
BH4
L-Citrulline
O_2^-
+
ONOO⁻
NO


GTP
GTPCH
DHFR
BH2
BH4
Oxidative Stress
NADPH
NADP⁺
O_2
NO
Coupled NOS
Arginine
Citrulline
GTP
GTPCH
DHFR
BH2↑
BH4↓
Oxidative Stress↑
NADPH
ONOO⁻
O_2
O_2^-
NO↓
Uncoupled NOS
Arginine↓
NADP⁺
ADMA↑
L-NMMA↑
GSSG:GSH↑


GTP
GTPCH
Tetrahydrobiopterin (BH4)
Oxidative stress
7,8-Dihydrobiopterin (BH2)
DHFR
MTX
BH4
eNOS
BH2
NO
O_2^-
Coupled
Uncoupled

Arteriosclerosos, Thrombosis and Vascular Biology, vol. 26, no. 12, pp. 2585–2587, 2006

Model for how the neutrophil could cause eNOS uncoupling.



Toll-Like Receptors (TLRs) and Damage-Associated Molecular Pattern (DAMPs).

TLRs:

- ✓ Transmembrane, pattern recognition receptors (PRR) (10 recognized subtypes in humans)

DAMPs:

- ✓ Endogenous molecules that may initiate immune response
- ✓ Act as proinflammatory mediators (alarmins)
- ✓ They are presented to immune system after **cellular lysis**, scheduled exocytosis, or after the release of enzymes' matrix

✓ TLRs also recognize pathogen-associated molecular pattern (**PAMPs**) (peptidoglycan & LPS pathogens)

✓ Macrophages, endothelial cells, dendritic cells, and lymphocytes express TLRs

✓ Kidney ***mesangial*** and ***tubular epithelial cells*** express TLR1, TLR2, TLR3, TLR4, and TLR6

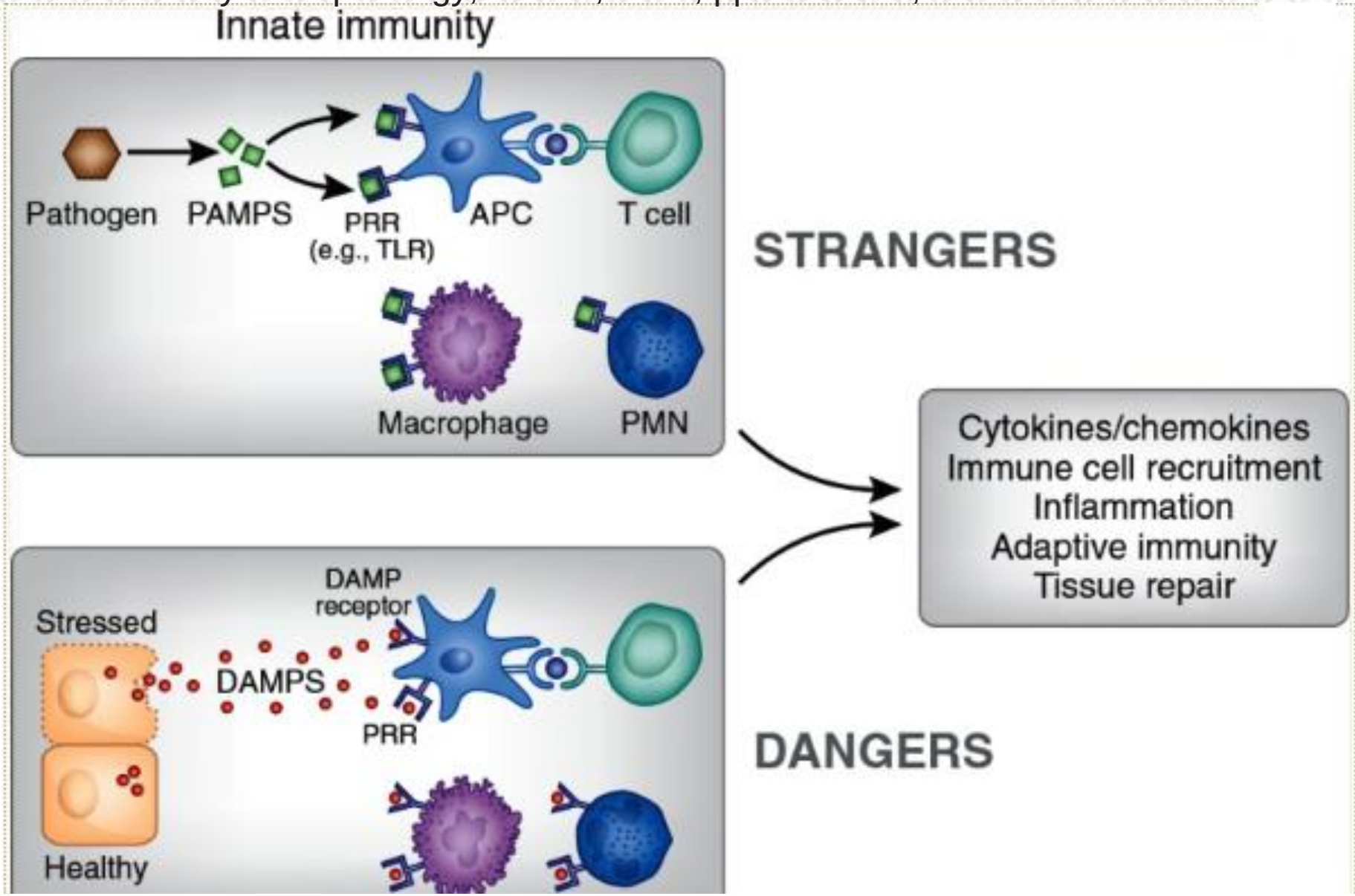
DAMPs are the triggering factors for *"danger alarm"* to fire innate immune response and come from endogenous, damaged cells, usually including proteins.

In AKI, heat shock proteins (*HSPs*) and high-mobility group box-1 (*HMGB-1*) protein are the most common

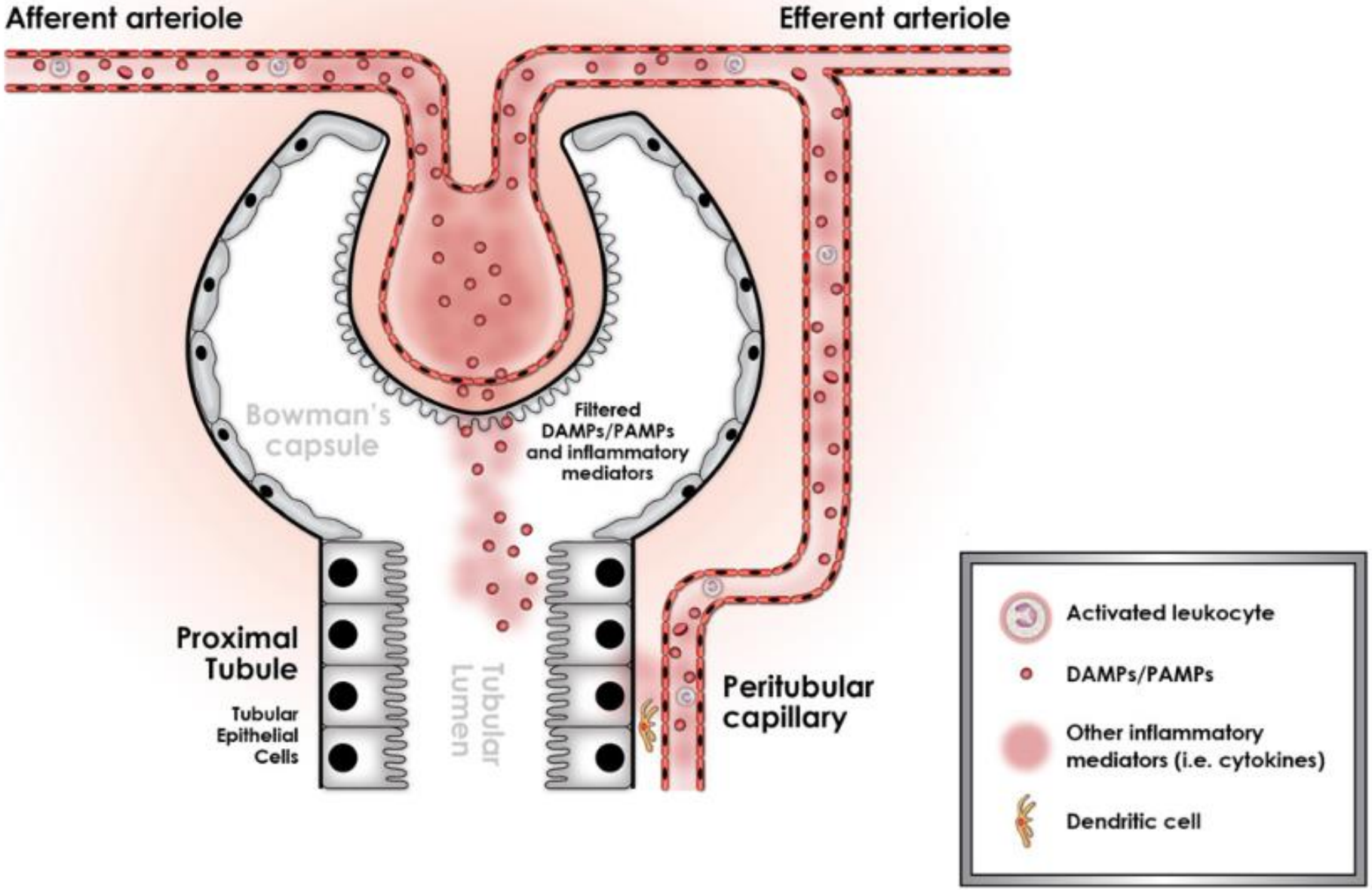
Once a ligand is bind on the receptor:

- ✓ With factors such as **MyD88** (myeloid differentiation factor 88) and **TRIF**(toll-receptor activator of interferon), endogenous pathways are activated:
  - ✓ Nuclear factor kappa-B (NFKB)
  - ✓ Mitogen-activated protein kinase pathway (MAPKP)
- ✓ Result in inflammation and *interferon production*

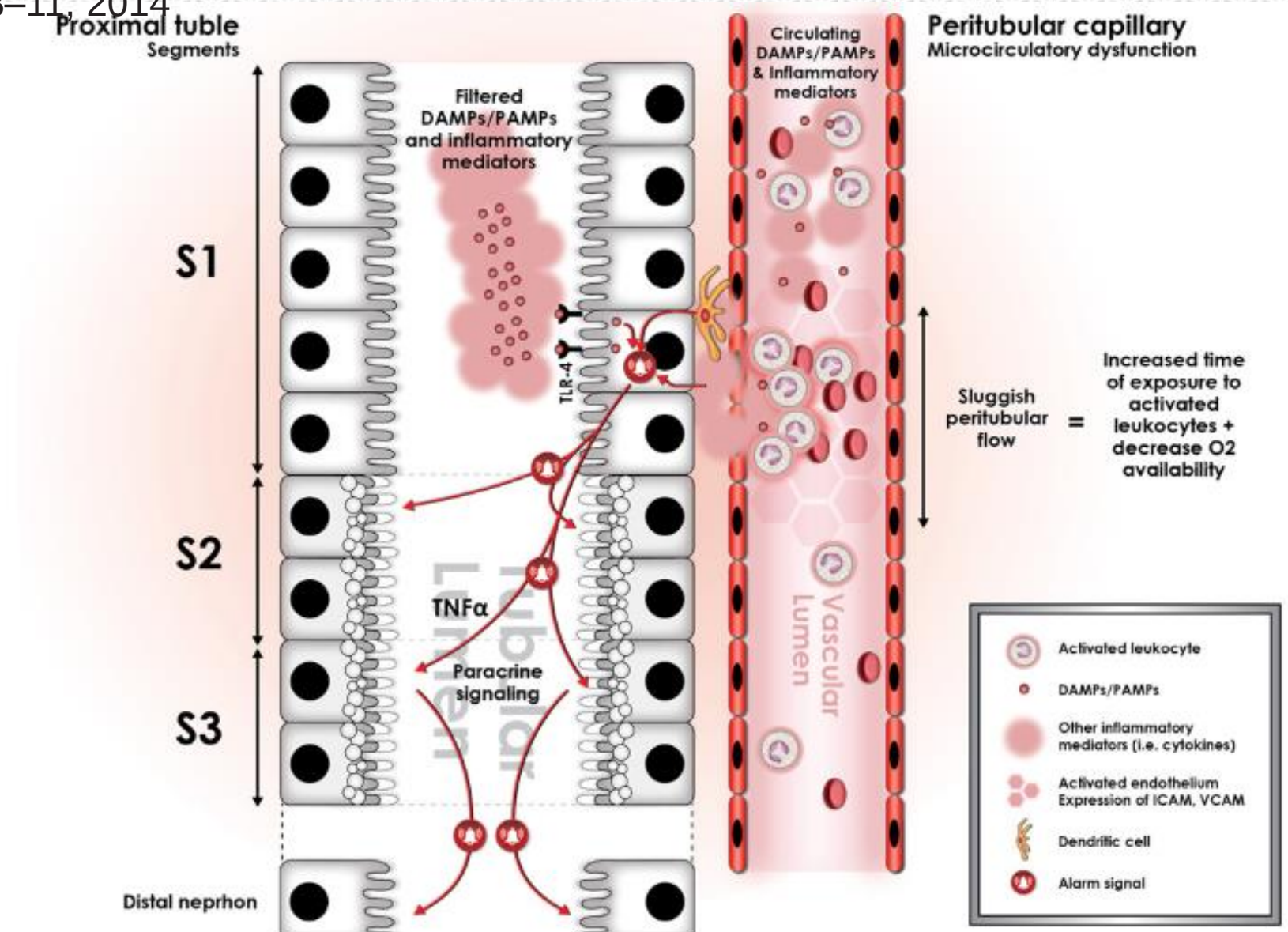
Journal of the American Society of Nephrology, vol. 22, no. 3, pp. 416–425, 2011



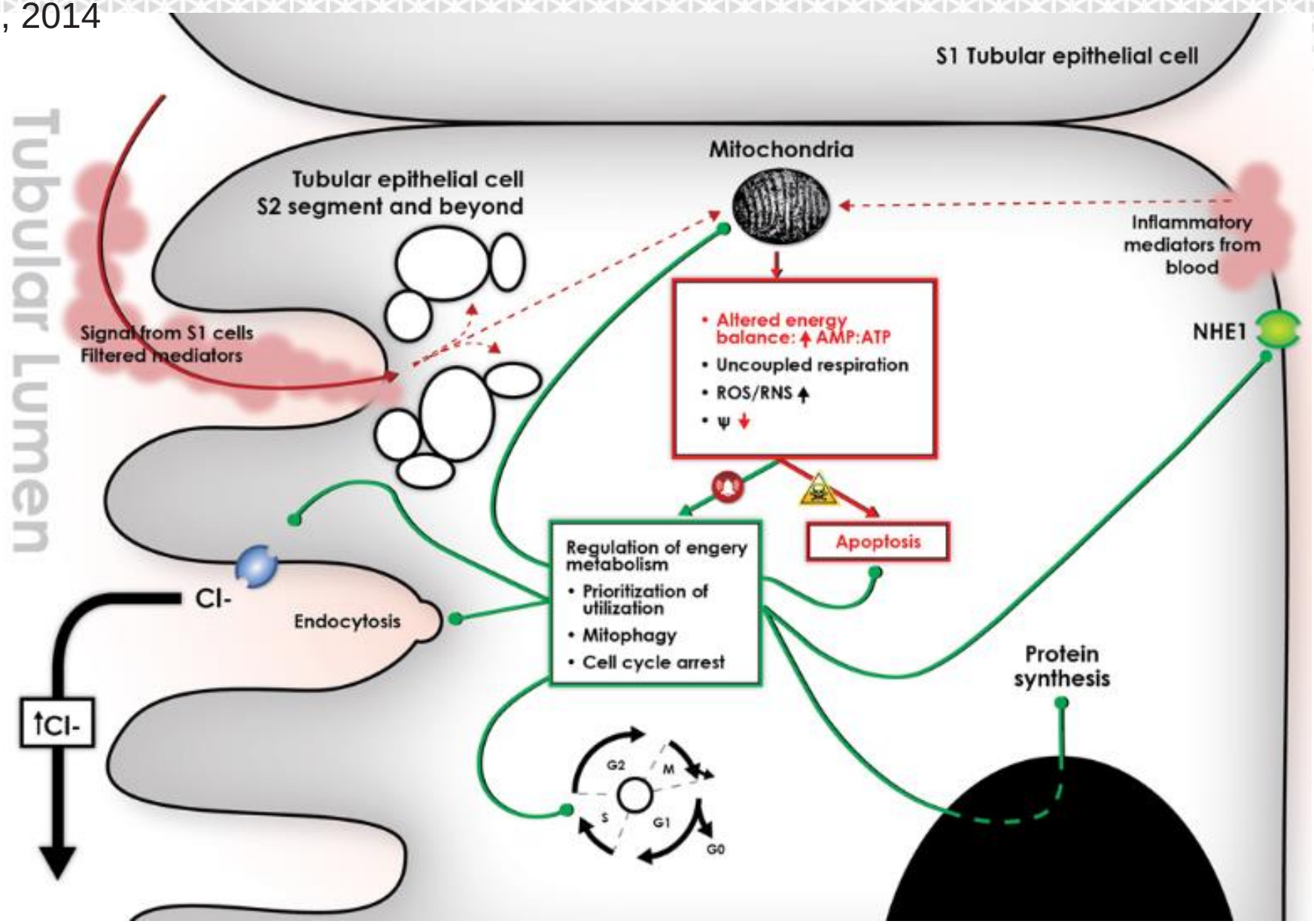
Shock, vol. 41, pp. 3–11, 2014



Shock, vol. 41, pp. 3–11, 2014

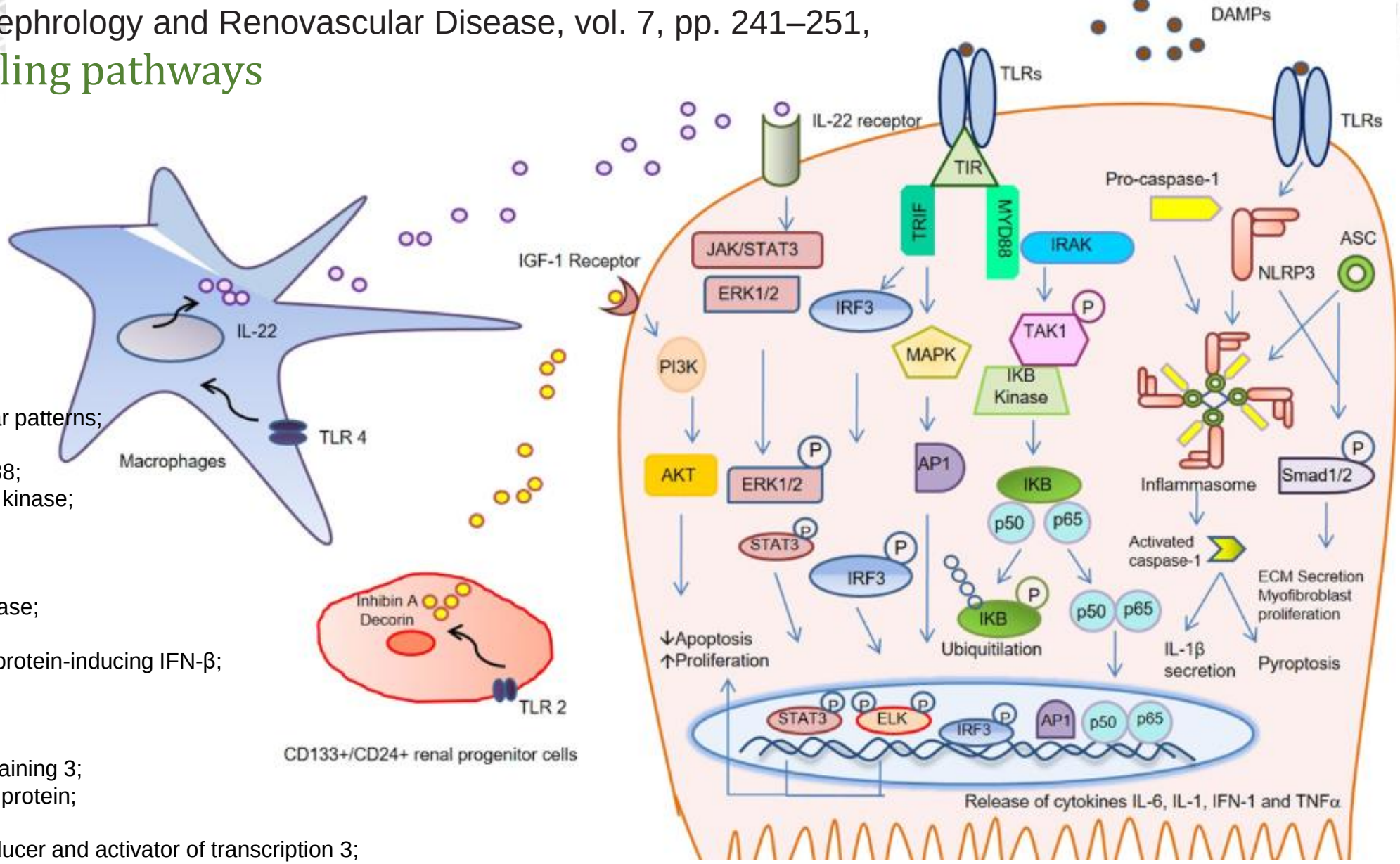


Shock, vol. 41, pp. 3–11, 2014



International Journal of Nephrology and Renovascular Disease, vol. 7, pp. 241–251, 2014

TLRs multiple signaling pathways



TLRs, Toll like receptors;
DAMPs, damage-associated molecular patterns;
TIR, Toll/IL-1β receptor;
MyD88, myeloid differentiation factor 88;
IRAK, interleukin receptor–associated kinase;
IκB, inhibitor of kappa B;
NF-κB, nuclear factor kappa B;
AP-1, activating protein-1;
MAPKs, mitogen-activated protein kinase;
IRF3, interferon regulatory factor 3;
TRIF, TIR domain containing adaptor protein-inducing IFN-β;
IL, interleukin;
INF, interferon;
TNF-α, tumor necrosis factor alpha;
NLRP3, NLR family pyrin domain containing 3;
ASC, apoptosis-associated speck-like protein;
IGF, Insulin-like growth factor;
Jak/STAT3, janus kinase-signal transducer and activator of transcription 3;
ERK, extracellular-signal-regulated kinase.

Autophagy in AKI.

- ✓ A continuous, catabolic process conserved through evolution, as a *"housekeeping"* process
- ✓ Aims at removal of **damaged and dysfunctional molecules**
- ✓ Enhanced response to acute situations such as **nutrient deficiency**
- ✓ Ensuring recycling of components for ***protein and energy synthesis*** and
- ✓ ***Elimination of toxic material***

ATG (autophagy-related genes) proteins increase in AKI

ATG proteins that augment in AKI with tubular dysfunction are **microtubule-associated protein light chain 3 (LC3)** and **Beclin-1**

Steps of Autophagy Process:

1. Formation of a double-membrane organelle called *phagophore.*
2. Phagophore sequesters the target, turning into an autophagosome.
3. Autophagosome fuses with lysosome to form autolysosome.
4. Lysosomal enzymes degrade cytoplasmic components for *recycling.*

Mitophagy.

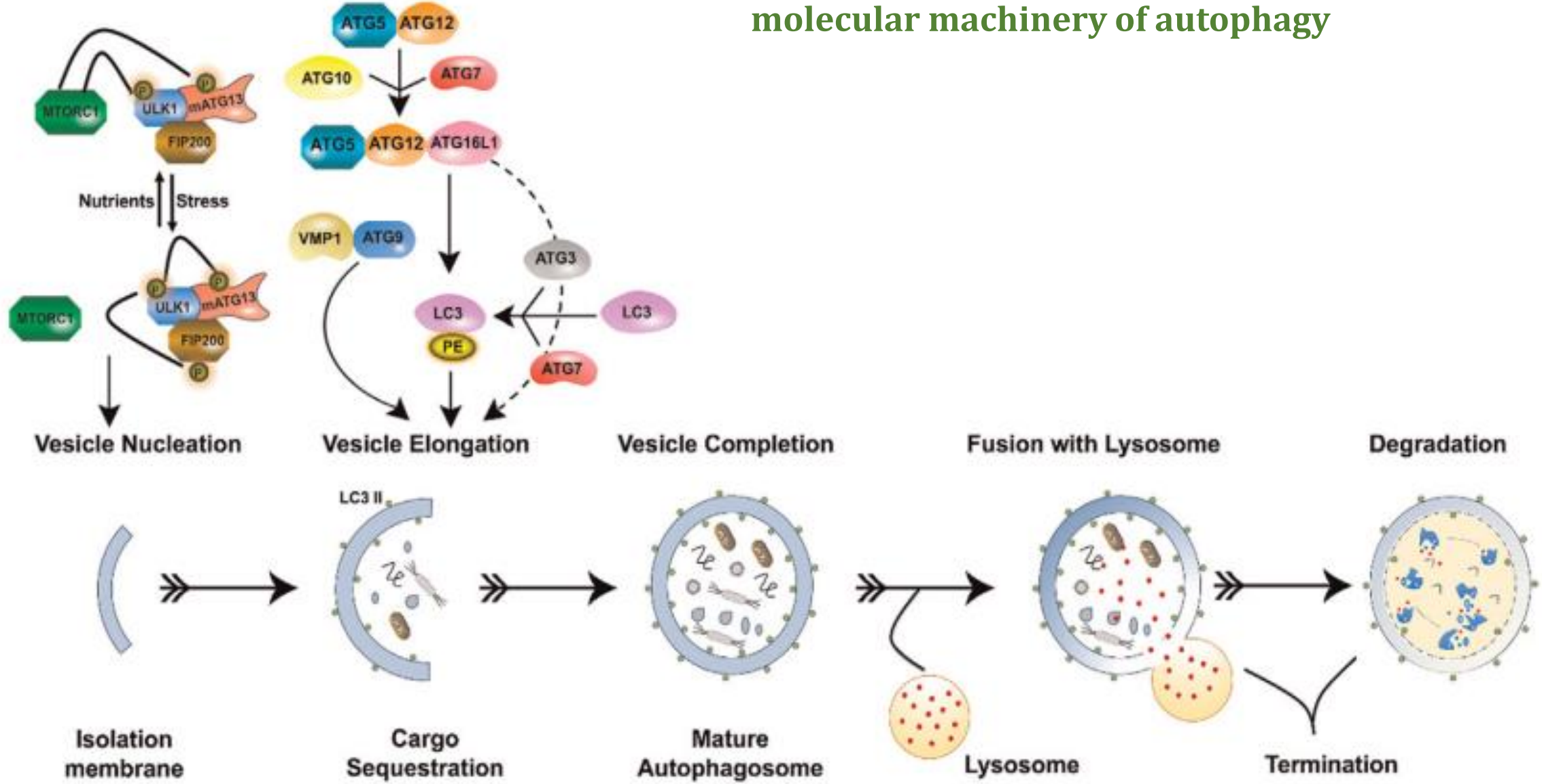
- ✓ In AKI, **hypoxic damage in tubular epithelial cells** is a potent stimulus for autophagy that is generally considered ***beneficial and nephroprotective***, preventing further structural compromise, especially at the **S3 segment** of the proximal tubule that is vulnerable to oxygen deprivation.

- ✓ In AKI, apart from hypoxia, the increased ROS production due to inflammation and oxidative stress causes *mitochondrial depolarization and dysfunction* lead to selective mitochondrial autophagy **("mitophagy").**

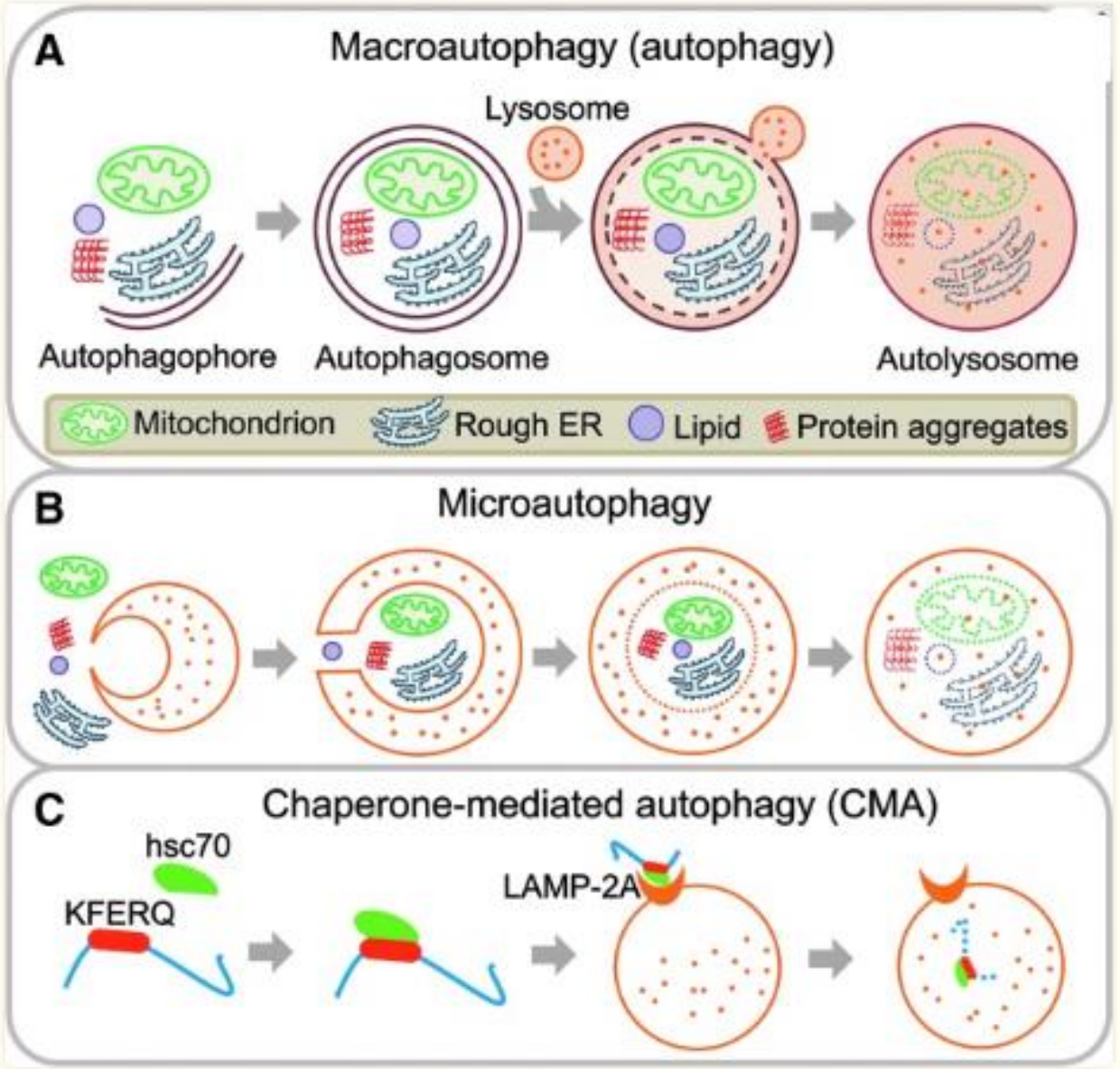
Contradictory opinions exist and claim that autophagy can be deleterious promoting cellular apoptosis, adding on to the renal injury.

Redox Biology, vol. 4, pp. 208–214, 2015

molecular machinery of autophagy



Antioxidants and Redox Signaling, vol. 40, no. 3, pp. 519–537, 2014



Microvascular Dysfunction.

Normally, outer medulla (10–20 mmHg) is perfused less than cortex (50 mmHg):

- ✓ Outer medulla is vulnerable zone to circulatory disturbances and hypoxia

Existence of focal hypoxemic renal tissue in AKI and oxygen deprivation:

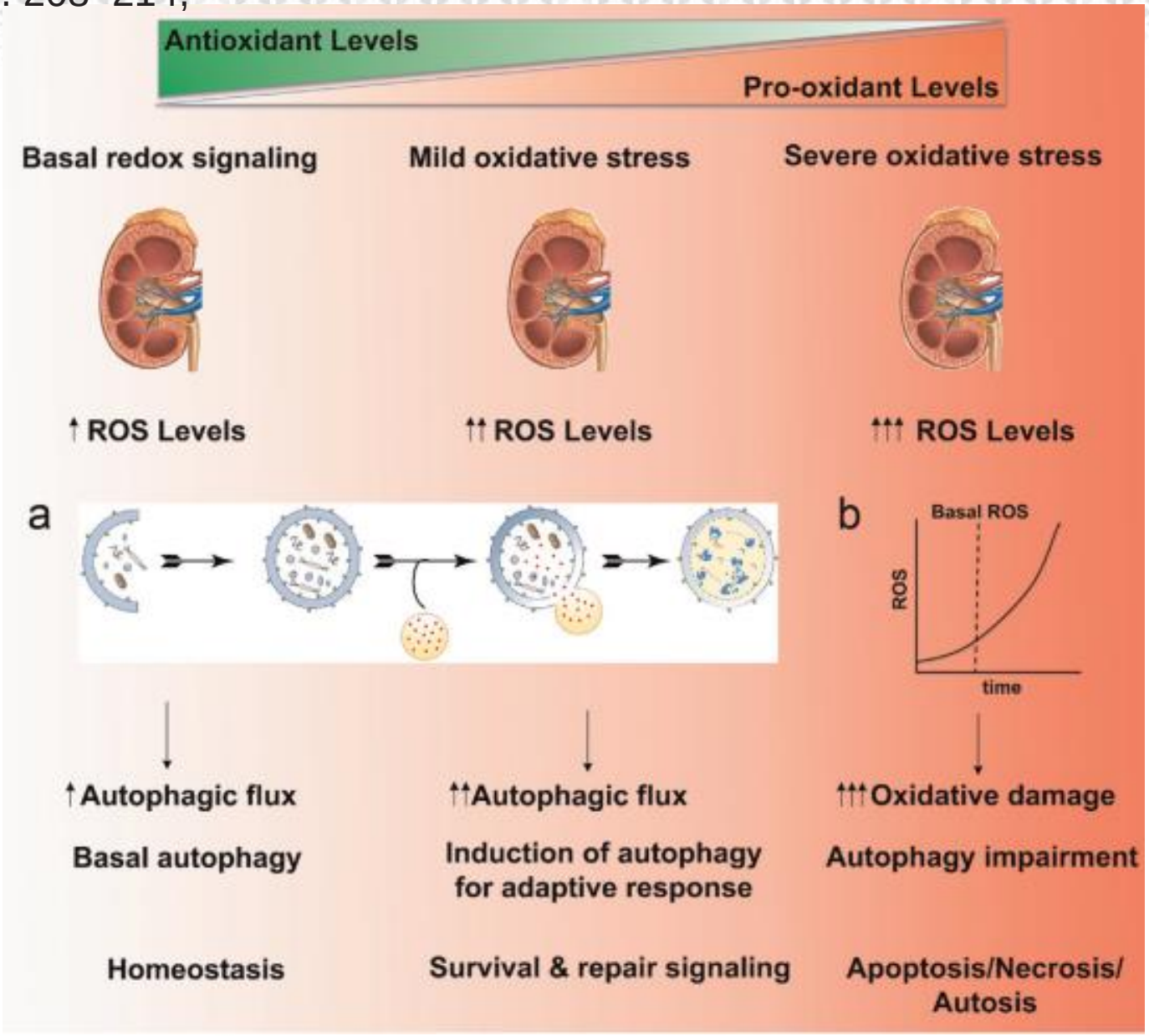
- ✓ **Anaerobic glycolysis** is enhanced, **lactic acid** is accumulated, **mitochondrial dysfunction** is enhanced, and production of **ROS** and superoxide is upregulated

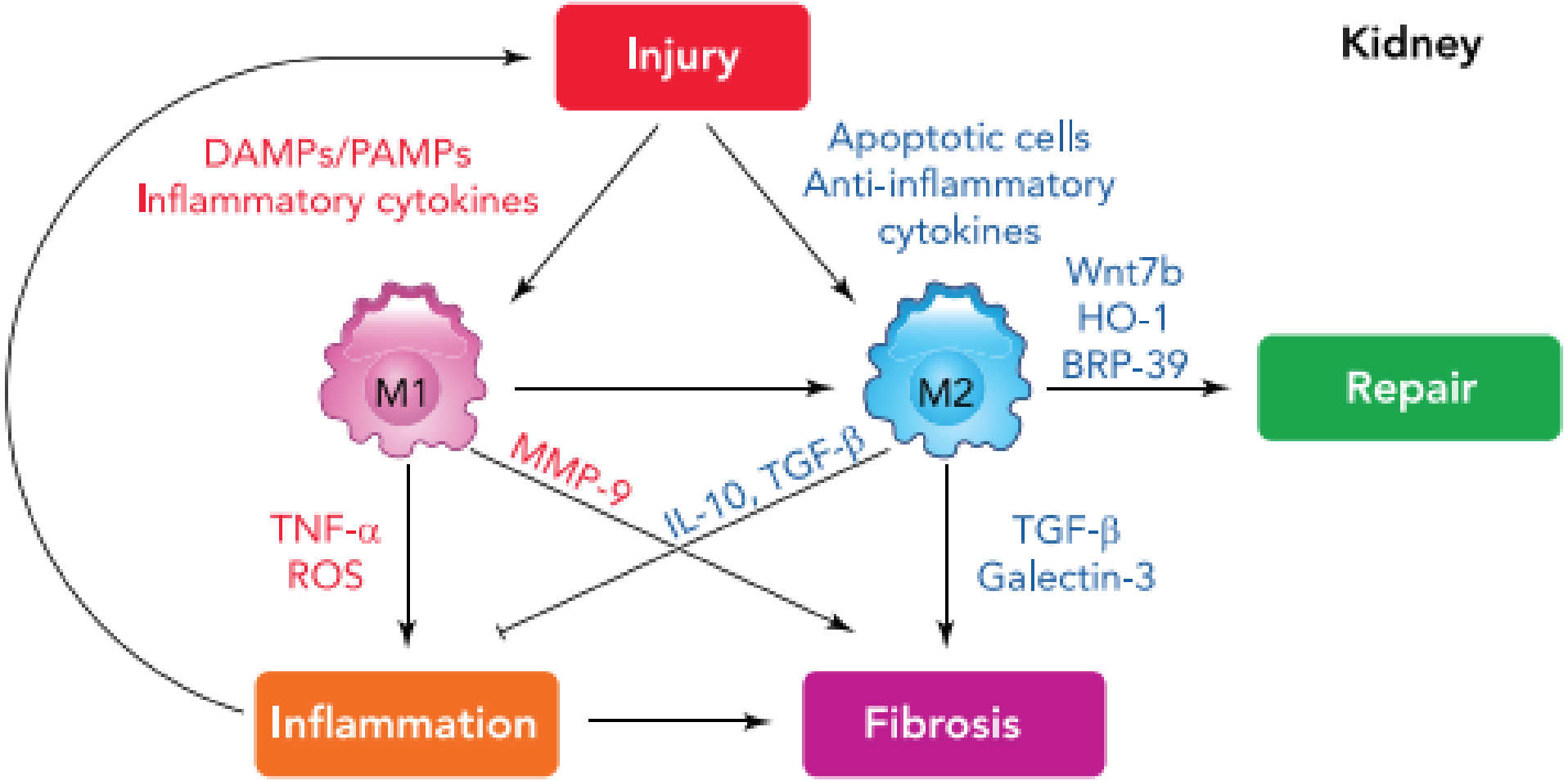
Injury expands after *reperfusion* that is characterized by:

- ✓ Inflammatory response with **leukocyte and complements activation** that
- ✓ Progresses to an oxidant environment that
- ✓ Can not be counterbalanced by antioxidant mechanisms
- ✓ and uneventfully leads to ***excessive cell death***

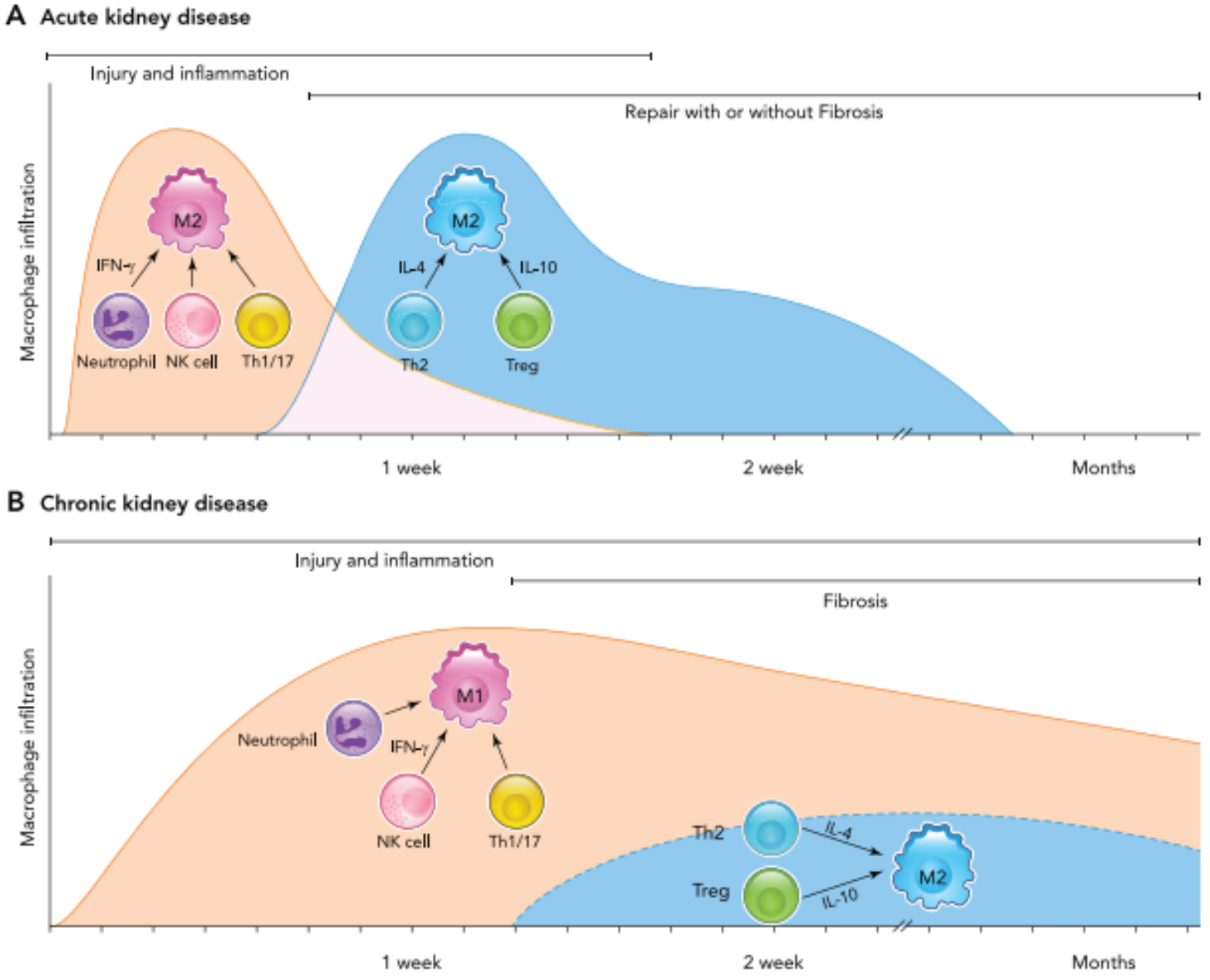
Endothelium holds a crucial role regarding the **expansion of inflammation**, through expression of **adhesion molecules** such as selectins , intracellular adhesion molecule-1 (ICAM-1), and CX3CL1 (fractalkine) that regulate inflammatory cell recruitment.

Redox Biology, vol. 4, pp. 208–214, 2015




Kidney
Injury
DAMPs/PAMPs
Inflammatory cytokines
Apoptotic cells
Anti-inflammatory
cytokines
Wnt7b
HO-1
BRP-39
M1
M2
Repair
MMP-9
IL-10, TGF-β
TNF-α
ROS
TGF-β
Galectin-3
Inflammation
Fibrosis

Physiology (Bethesda) 2015; 30: 183–194



Ischemic preconditioning (IPR):

- ✓ Was introduced in 1986 by Murry et al. in an animal model that **sustained brief ischemic episodes** before a major ischemic event resulted in a beneficial outcome for the organ.
- ✓ In 1993, Przyklenk et al. described a slightly different model of ischemic preconditioning (**remote and rIPR)** that has been further modified and is currently followed, when the direct approach to the involved organ is not feasible.

In brief, after the main stimuli (ischemia) is withdrawn, a series of responses take place (**neural, humoral pathway, and systemic anti-inflammatory response**) with the final receiver being the **mitochondrion.** The subsequent **opening of the ATP dependent mitochondrial potassium channel** prevents the opening of the mitochondrial permeability transition pores (**MPTP**) that enhances the **stability of its membrane and the survival after IRI.**

Prediction of AKI by Oxidative Stress Biomarkers in Critically Ill Patients.

Oxidative stress can be assessed by indirect methods which can measure the *stable by-products of ROS activity* on biomolecules. In the setting of critical illness, the most commonly measured markers of oxidative stress are:

- ✓ *Isoprostanes, hydroxynonenal and lipid peroxides, chlorinated compounds, oxidized glutathione, nitrated and oxidized proteins, and malondialdehyde detected as thiobarbituric acid reactants (TBARs).*

- ✓ **Plasma** levels of **F2-isoprostanes** and **isofurans**
- ✓ **Urine** Liver-type fatty acid-binding protein (**L-FABP**): important *cellular antioxidant* by maintaining low levels of free fatty acids in the cytoplasm of tubular cells through facilitation of intracellular metabolism and excretion in urine
- ✓ **Erythrocyte** superoxide dismutase (**SOD1**) activity

- ✓ **Plasma and urine** neutrophil gelatinase-associated lipocalin (**NGAL**): released from the **injured *distal nephron***, ability to *scavenge iron*, bacteriostatic effects, protection against oxidative stress damage, sensitivity of 81–96% and specificity of 51–68% for prediction of AKI

- ✓ Upregulation of endogenous **antioxidants** such as **SOD1** and **SOD2** as well as **HO1** levels

- ✓ **Urinary** kidney injury molecule-1 (**KIM-1**) and interleukin-18 (**IL-18**) are suggested as good markers for the **prediction of progressive AKI**

What should be mentioned is that NGAL, IL-18, and KIM-1 are **inflammatory mediators** that increase in inflammatory situations regardless of the presence of AKI and are indivisible parameters concerning their assessment in the prediction of AKI.

TIMP-2 **(***tissue inhibitor of metalloproteinase 2)* and ***IGFBP-7*** **(***insulin-like growth factor-binding protein 7):*

- ✓ *Predictive urine biomarkers of AKI in high-risk patients*
- ✓ ***Cell cycle arrest biomarkers*** (G1 cell cycle arrest phase)
- ✓ During **very early stages** of cellular stress
- ✓ Urine product of TIMP-2 and IGFBP-7 is *superior in the prediction of KDIGO stage 2-3 AKI* (SAPPHIRE, OPAL study)
- ✓ Cut-off values for risk stratification of AKI with high-risk patients when *TIMP-2 × IGFBP-7 is over 0.3*
- ✓ *Highest risk for patients with product value is over 2*

In **persisting AKI** that is equal with the ongoing damage:

- ✓ Levels of TIMP-2 × IGFBP-7 product remain elevated
- ✓ Indicating the maintenance of cell cycle arrest (in G1 phase)
- ✓ May uneventfully lead to **failure of recovery and renal fibrosis**

Clinical Evidence in AKI Prevention by Targeting Oxidative Stress.

Existing clinical evidence comes from small cohorts and studies

Scavenging of free radicals in order to avoid the provocation of chain reactions:

- ✓ **Anesthetics: SOD mimetics** (sodium pentothal and propofol) and lidocaine
- ✓ **N-acetylcysteine (NAC):** scavenger of **OH-** mainly, administered **parenterally** (bioavailability is low orally), induction of glutathione synthesis, reduces the incidence of AKI after **contrast media** administration, but direct intravenous administration of glutathione is better
- ✓ **HMG-CoA reductase inhibitors (statins):** vascular endothelium function preservation through **upregulation of eNOS**, increasing the available NO and contribute to the restriction of free radical generation from lipids' oxidation, protection of renal function after **PCI, ACS, IRI.**

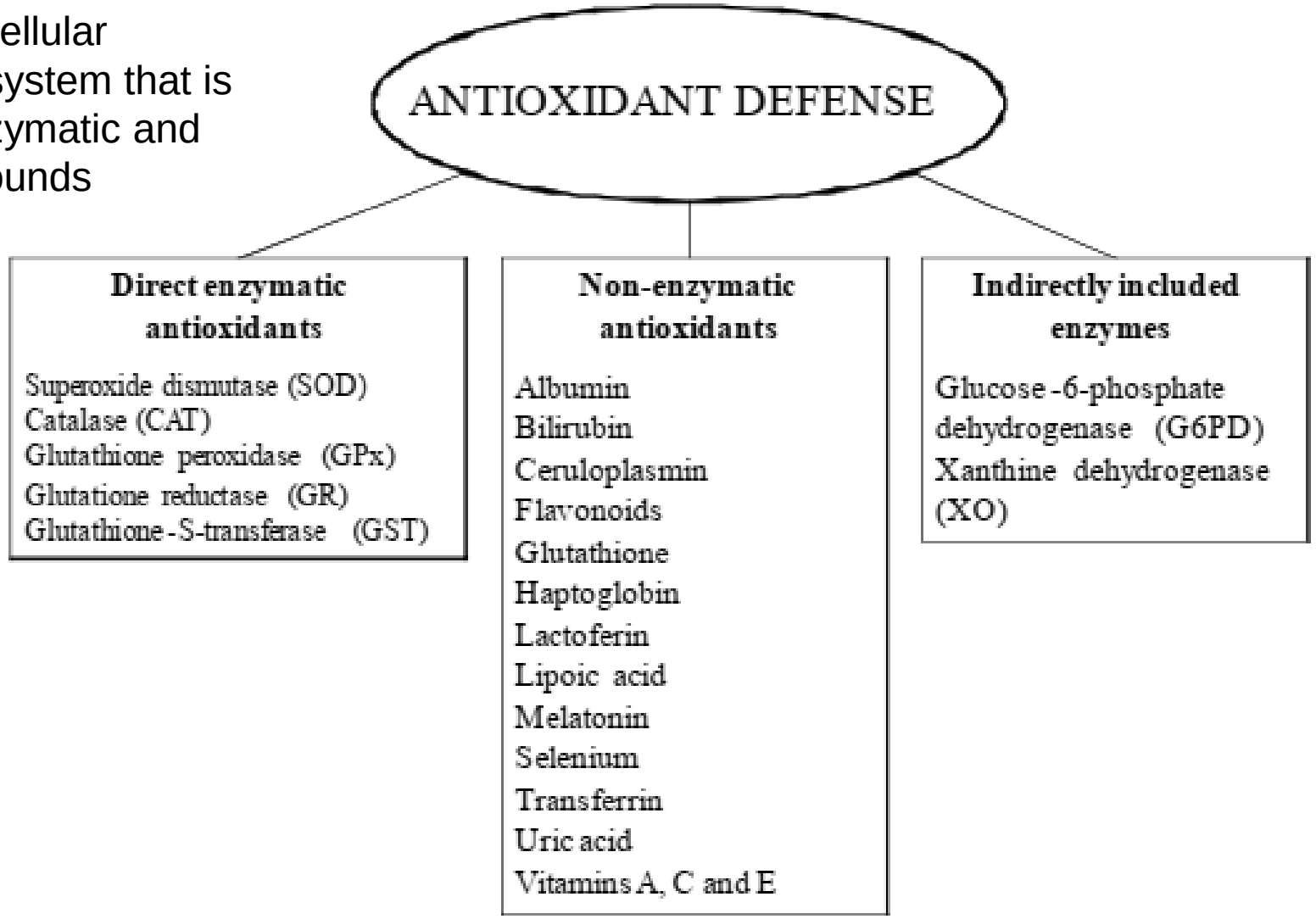
Therapeutic Interventions and Future Perspectives.

- ✓ **Low-dose dopamine** in continuous infusion: temporary benefit in urine output, no significant protection against development of AKI
- ✓ **Fenoldopam**: superior compared to dopamine (reduced need for RRT and ICU)
- ✓ Early recommencement of enteral versus parenteral feeding in ICU patients (even before 48 hours of hospitalization): maintenance of normal intestinal microflora, better survival and less infections
- ✓ Supplementation of vitamins and trace elements: thiamine, vitamin A, C and E, and selenium; improve survival and reduce infectious

In general, the substitution of more than 66% of the recommended daily allowance of vitamins A, C, and E has been shown to improve antioxidant capacity

- ✓ Enteral administration of melatonin and parenteral administrations of NAC plus deferoxamine have been correlated with better **total antioxidant capacity (TAC)** in serum.

The components of cellular antioxidant defense system that is consisted of both enzymatic and nonenzymatic compounds



Conclusions.

Acute kidney injury is a multifactorial clinical entity representing a major health problem.

In critical care, AKI remains highly prevalent, complicating the clinical course of patients, extending the need for ICU hospitalization, requiring RRT, and carrying high mortality.

Mechanisms of oxidative stress involved in AKI summarize to:

- ✓ **ROS generation**
- ✓ **NO depletion**
- ✓ **DAMP generation and TLR activation**
- ✓ **Autophagy**
- ✓ **Microvascular dysfunction**

These mechanisms **prevail over endogenous antioxidants and regulatory mechanisms** so that physiological homeostasis is abolished and AKI is finally installed.


THANK YOU