Inflammation and Complement System in AKI

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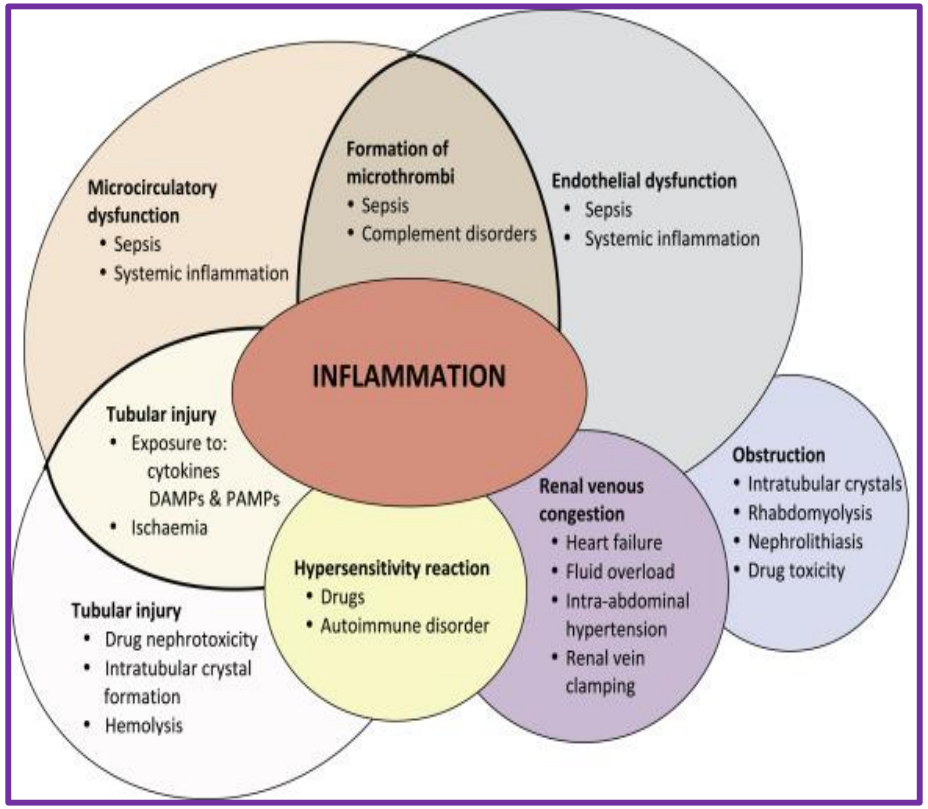
Outline

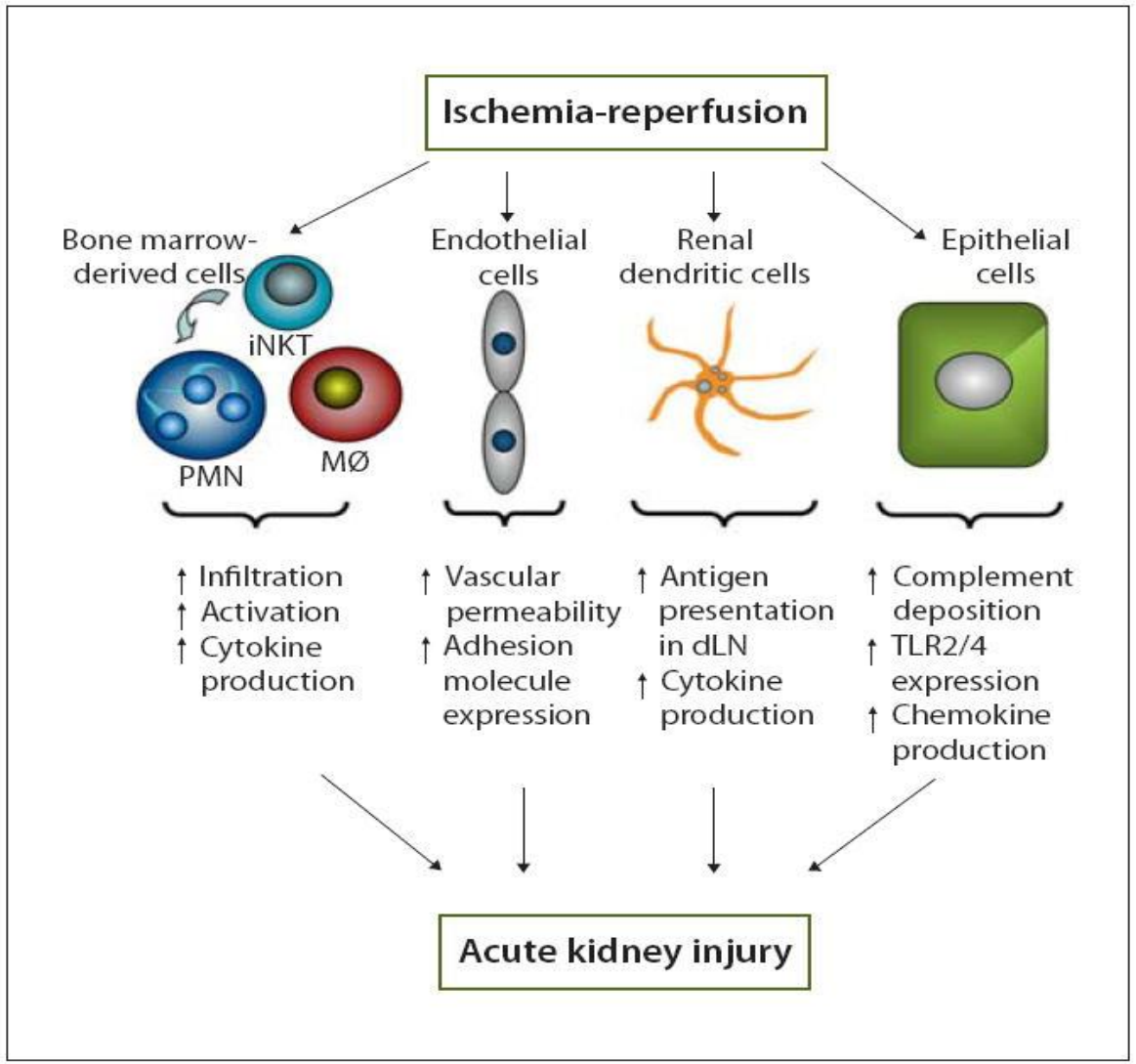
- ❑ Inflammatory pathways in AKI
- ❑ Complement system in AKI
- ❑ Maladaptive repair and AKI-CKD transition
- ❑ AKI biomarkers and therapeutic options on the horizon

Acute Kidney Injury (AKI) Etiology and Prognosis

- Most AKI is **intrinsic** in nature, associated with **ischemia, sepsis or nephrotoxins** and complicated with **high morbidity/mortality.**
- **Ischemia/reperfusion (I/R) is a common cause of AKI** after kidney transplantation, cardiac surgery.
- The pathogenesis of intrinsic AKI involves a **cross talking between renal system and the innate and adaptive immunity**.
- If the immunopathologic processes in AKI continue, this can lead to **renal fibrosis, CKD/ESRD, cardiovascular disease and mortality**.



Marlies Ostermann M. Best Practice & Research Clinical Anaesthesiology 2017



Gilbert R. Kinsey, Li Li, and Mark D. Okusa

Nephron Exp Nephrol. 2008.

AKI is characterised by an exacerbated inflammatory response: different cells, inflammatory mediators oxidative processes and complement system play a role.

Cellular and Pathopysiological Mechanisms of AKI

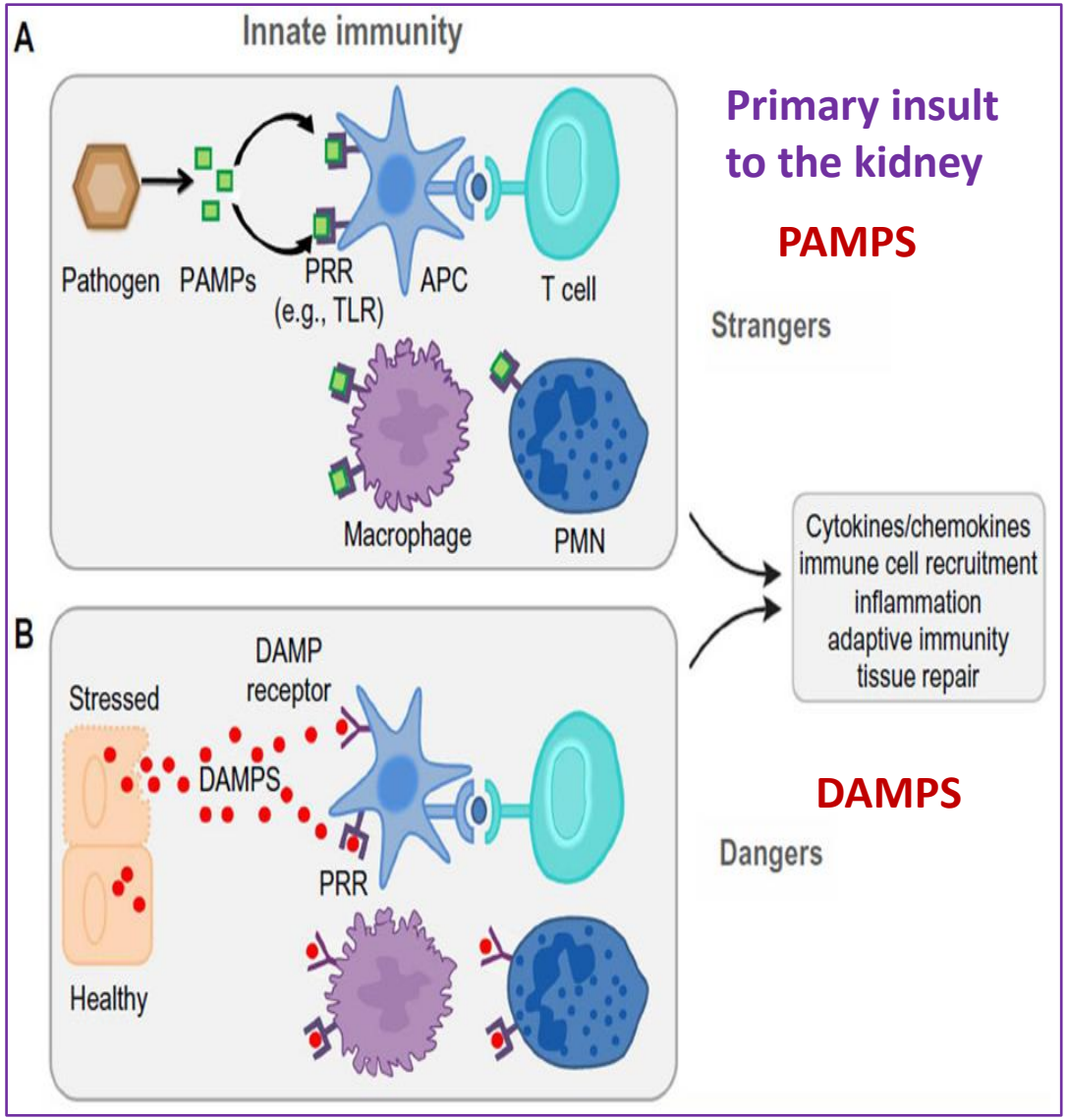


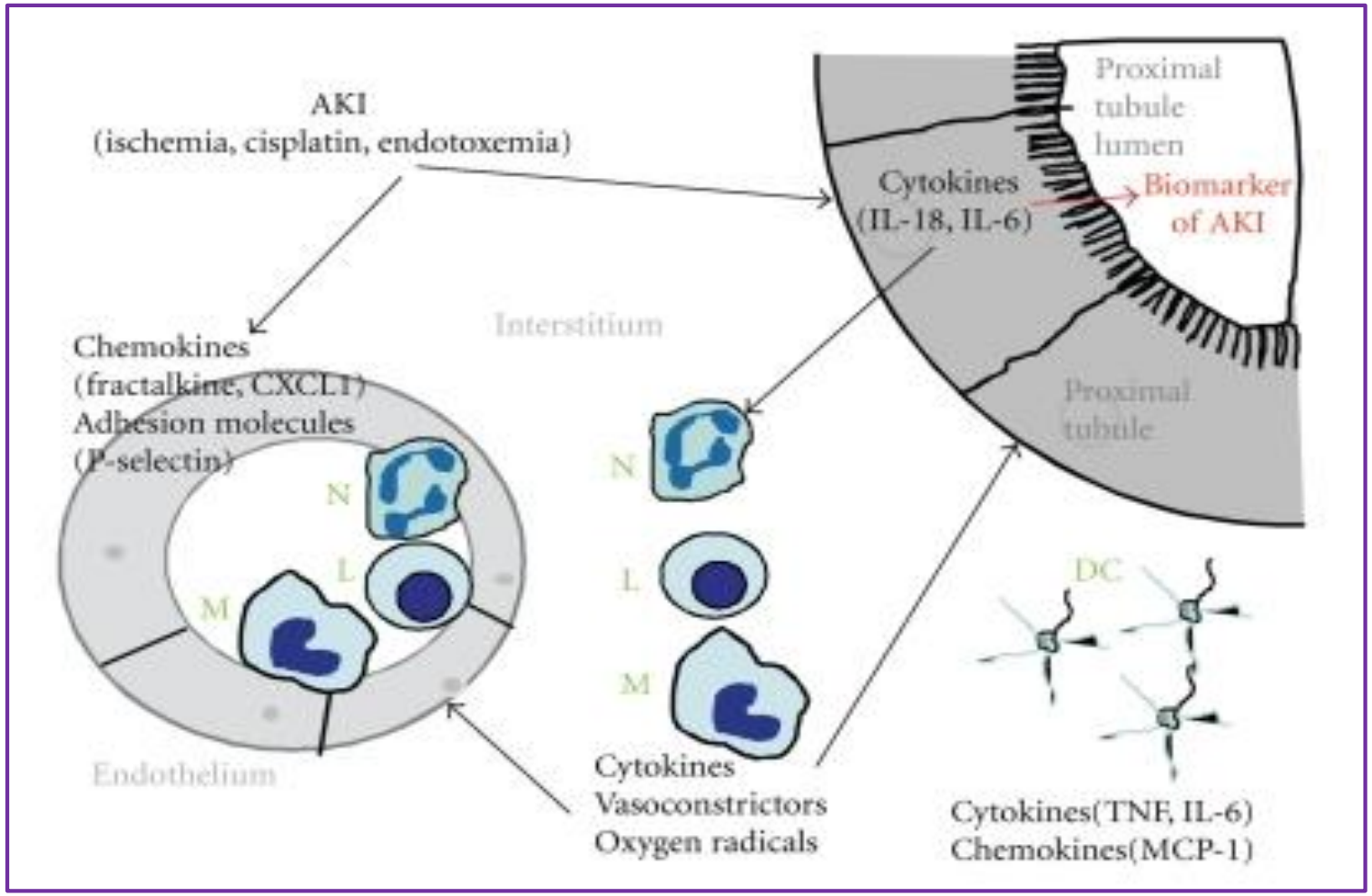
Table 1. Innate and Adaptive Cellular Immunity Factors in Intrinsic Acute Kidney Injury Pathogenesis.

Immune System Factor	Cellular and Molecular Pathways
Pathogen-associated molecular patterns	Lipopolysaccharides (LPS), pattern recognition receptors (PRRs), and renal tubular epithelial (RTE) cells
Damage-associated molecular patterns	PRRs and RTEs
Toll-like receptors (TLRs)	LPS-LBP-CD14-TLR complex, MyD88, and RTEs
Mitochondria	Reactive oxygen species (ROS), mitochondrial proteins, and mitochondrial biogenesis
Cell death	Necroptosis, receptor-interacting protein 1, receptor-interacting protein 3, and mixed lineage kinase domain-like protein
Hypoxia inducible factor	Oxygen delivery, vascularization, neoangiogenesis, vascular tone, glucose metabolism, and anaerobic glycolysis
Complement system	Cell surface and soluble complement regulatory proteins
Adhesion molecules	Endothelial cells, selectins, and integrins
Dendritic cells (DCs)	Resident renal DCs
M1 and M2 macrophages	Pro-inflammatory and anti-inflammatory cytokines and chemokines
Neutrophils	ROS and leukotriene B4
B and T lymphocytes	RTEs, adhesion molecules, Th17, Th1, and Th2 cytokines
Natural killer cells	Bridge innate and adaptive immunity

Note. *LBP = LPS-binding protein;* MyD88 = myeloid differentiation primary response 88; *Th1* = type *1 T helper; Th2* = type 2 *T helper; Th17* = type *17 T helper.*

Radi ZA. Toxicologic Pathology 2018, Vol. 46(8) 930-943

Proximal RTE is most sensitive to the injury: Victim or Foe?



AKI results in the upregulation of chemokines like fractalkine and CXCL1 and adhesion molecules like P-selectin in the endothelium of blood vessels in the kidney. Upregulation of chemokines and adhesion molecules in the endothelium results in the infiltration of inflammatory cells like neutrophils (N), lymphocytes (L), and macrophages (M) from the blood vessels into the interstitium of the kidney. Proximal tubular epithelial cells produce cytokines like IL-18 and IL-6. IL-18 and IL-6 produced by the proximal tubule enter the interstitium and result in activation and/or proliferation of neutrophils (N), lymphocytes (L), and macrophages (M). IL-18 and IL-6 are also released from the proximal tubular cells into the tubular lumen Inflammatory cells produce vasoconstrictors (prostaglandins, leukotrienes, and thromboxanes) that may affect vascular injury and oxygen radicals that may worsen tubular and vascular injury. Resident dendritic cells (DCs) form a contiguous network throughout the entire kidney. The role of DCs in AKI is not well understood. Following kidney ischemia-perfusion, resident dentritic cells release cytokines like TNF and IL-6 and chemokines like MCP-1.

Endothelial cells and tubular epithelial cells undergo morphological/ functional changes and produce cytokines.

Leukocytes (N, M, NK) and lymphocytes infiltrate kidney and become activated for **pro-inflammatory cytokine production** (TNF-α, IFN-γ, TGF-β, IL-1, etc)

Edelstein CL. Mediators Inflamm. 2009; 2009: 137072.

Table 1: Inflammatory mediators of AKI.

Caspases
Caspase-1
Alpha-MSH
Cytokines
(IFN-γ)
(TGF-β)
(TNF-α)
(IL-6)
(IL-10)
(IL-18)
Chemokines
(CXCL1 (also known as KC or IL-8 in mice))
(CX3CL1 (also known as fractalkine))
(CCR1 (Chemokine receptor-1) ligands MIP-1alpha and RANTES)
(MCP-1)
(MIP-2)
Adhesion molecules
(ICAM-1)
(P-selectin, E-selectin)
(CD147)
Complement
Alternative pathway
C3A
TLRs
TLR-2
TLR-4
TLR-9
Neutrophils
Lymphocytes
CD4
CD8
B cells
Tregs
NK cells
Macrophages
Dendritic cells

Edelstein CL. Mediators Inflamm. 2009; 2009: 137072.

Immune regulation of cytokines in AKI

- **Proinflammatory cytokines and chemokines:**
  - (TNF-α, IL-6, IL-18, IFN-γ, IL-20, *Caspase -1 induced* IL-1B and IL-18, MCP-1, CX3CL1)
- **Antinflammatory inhibitors and antagonists:**
  - (IL-10, IL-4, IL-13, CSF and GM-CSF)
- **Dysregulated mechanism and excessive cytokines is responsible for AKI immunopathogenesis.**

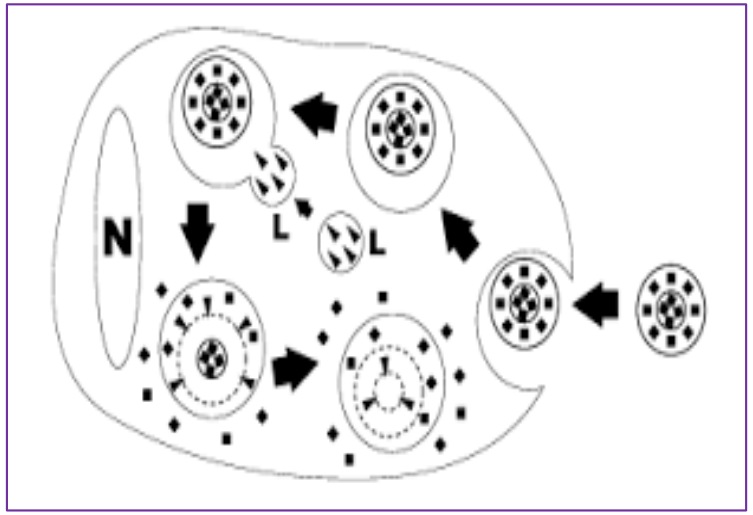
Am J Physiol Renal Physiol 294: F264–F271, 2008.
First published November 14, 2007; doi:10.1152/ajprenal.00204.2007.

Fractalkine receptor (CX3CR1) inhibition is protective against ischemic acute renal failure in mice

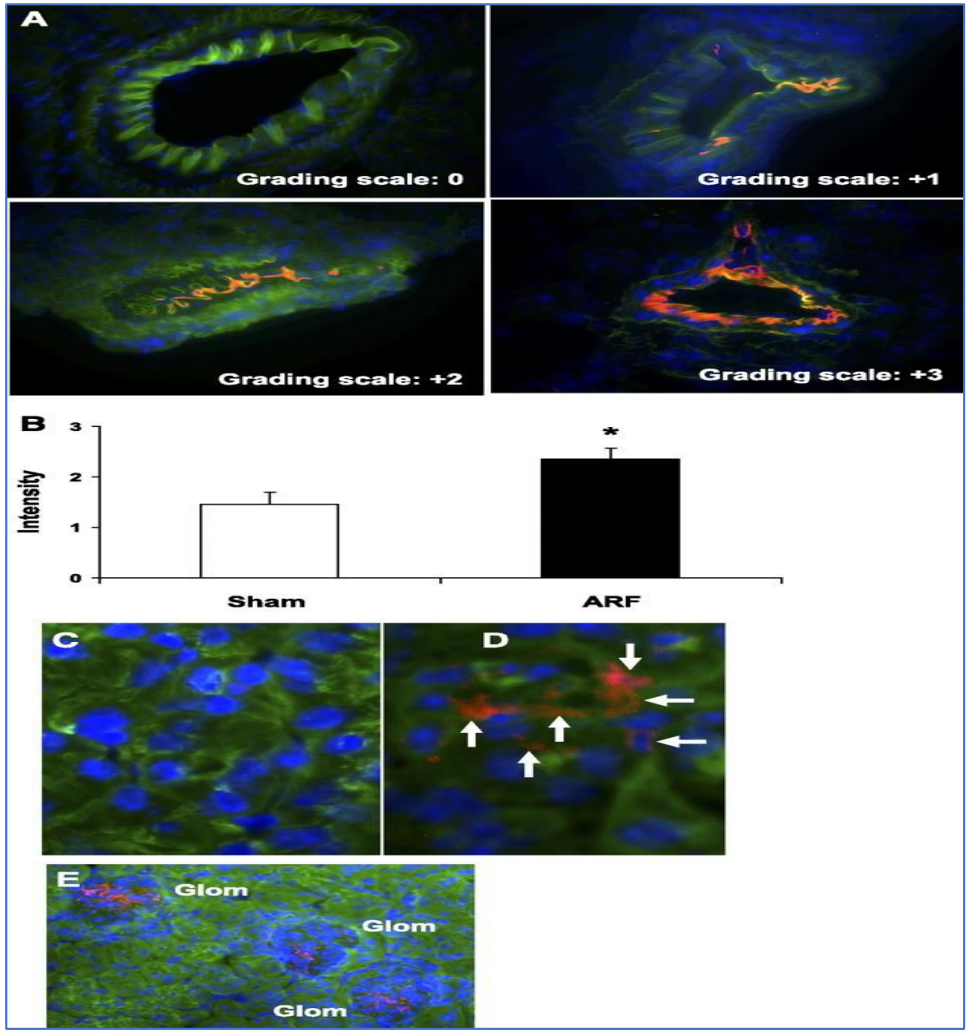
Dong-Jin Oh,[1]* Belda Dursun,[1]* Zhibin He,[1] Lawrence Lu,[1] Thomas S. Hoke,[1] Danica Ljubanovic,[2] Sarah Faubel,[1] and Charles L. Edelstein[1]

Oh D-J, Dursun B, He Z, Lu L, Hoke TS, Ljubanovic D, Faubel S, Edelstein CL. Fractalkine receptor (CX3CR1) inhibition is protective against ischemic acute renal failure in mice. *Am J Physiol Renal Physiol* 294: F264–F271, 2008. First published November 14, 2007; doi:10.1152/ajprenal.00204.2007.—Fractalkine (CX3CL1) is expressed on injured endothelial cells and is a potent chemoattractant and adhesion molecule for macrophages carrying the fractalkine receptor (CX3CR1). The aim of this study was to investigate the role of CX3CL1, and its ligand CX3CR1, in ischemic acute renal failure (ARF) in mice. On immunoblotting, CX3CL1 protein expression in the kidney increased markedly in ischemic ARF. On immunofluorescence staining, the intensity of CX3CL1 staining in blood vessels was significantly more prominent in ischemic ARF compared with controls. A specific anti-CX3CR1 antibody (25 μg ip 1 h before induction of ischemia) was functionally and histologically protective against ischemic ARF. CX3CR1 is predominantly expressed on macrophages. Macrophage infiltration in the kidney in ischemic ARF was significantly decreased after anti-CX3CR1 antibody treatment. To determine the role of macrophages in ischemic ARF, macrophages in the kidney were depleted using liposomal-encapsulated clodronate (LEC). LEC resulted in significant functional and histological protection against ischemic ARF. In summary, in ischemic ARF, *1*) there is upregulation of CX3CL1 protein in the kidney, specifically in blood vessels; *2*) CX3CR1 inhibition using a specific antibody is partially protective and is associated with reduced macrophage infiltration in the kidney; and *3*) macrophage depletion in the kidney is protective.

- **Fractalkine (CX3CL1) is expressed on injured endothelial cells** and is a **potent chemoattractant and adhesion molecule for macrophages carrying the fractalkine receptor (CX3CR1).**
- The aim of this study was to investigate:
  - **The role of CX3CL1, and its ligand CX3CR1, in ischemic acute renal failure (ARF) in mice.**
  - **Whether macrophage depletion is protective**



Macrophage depletion by liposome encapsulated clodronate (LEC)



CX3CL1 immunofluorescence staining. *A*: intensity of endothelial staining of CX3CL1 in arteries was graded from 0 to 3+. The red/orange color indicates positive staining for CX3CL1. Blue staining represents nuclei. Representative pictures of the grading scale are demonstrated. *B*: using the grading scale demonstrated in *A*, the intensity of endothelial staining was increased in ischemic ARF compared with sham-operated mice (*$P < 0.05$ vs. sham, $n = 4$). *C* and *D*: representative pictures of CX3CL1 staining (red/orange, arrows) in capillaries in the outer stripe of the outer medulla in sham-operated (*C*) and ARF (*D*) mice. *E*: CX3CL1 staining (red/orange) in capillaries in a normal glomerulus (glom) from a sham-operated mouse.

Fractalkine receptor (CX3CR1) expression is increased in ischemic AKI with strongest expression at vascular sites close to **macrophage accumulation.**

Inhibition of fractalkine receptor decreases macrophage infiltration and **protects against ischemic AKI**.

Macrophage depletion in the kidney (by liposomal encapsulated clodronate) **is protective .**

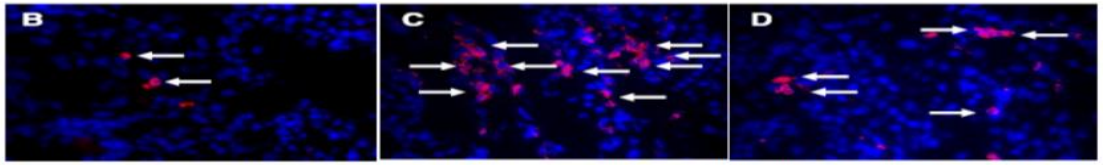
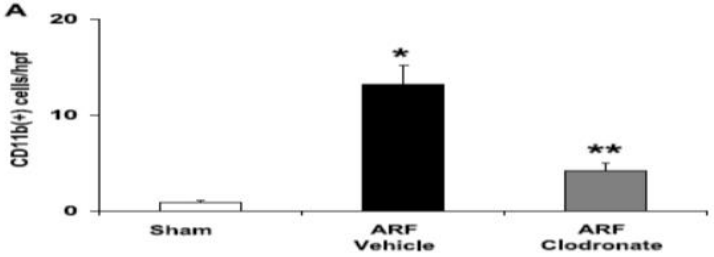


Fig. 5. Immunofluorescence staining for CD11b. *A*: the number of CD11b (+) macrophages was significantly increased in ischemic ARF compared with sham (*$P < 0.001$ vs. sham) and reduced by anti-CX3CR1 antibody treatment (**$P < 0.01$ vs. vehicle). *B*: representative pictures of immunofluorescence staining for CD11b-positive cells (arrows) in the outer stripe of outer medulla in sham-operated (*B*), ischemic ARF+vehicle (*C*), and ischemic ARF+Ab (*D*) are demonstrated.



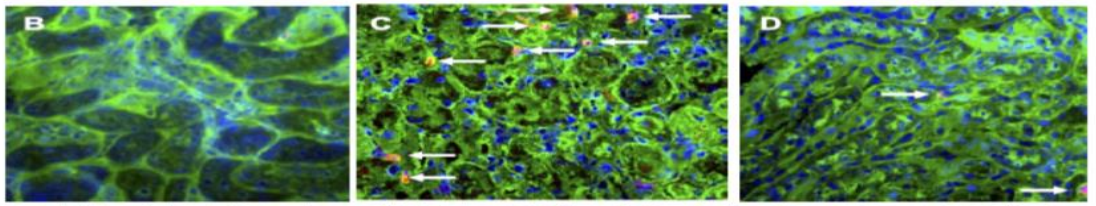


Fig. 6. Macrophage depletion studies. *A*: liposomal-encapsulated clodronate (LEC) resulted in a significant decrease in macrophage infiltration in the kidney in ischemic ARF. *$P < 0.01$ vs. sham. **$P < 0.05$ vs. ischemic ARF+vehicle. Empty liposomes prepared in the same manner as LEC were used as the vehicle. Representative pictures of the immunofluorescence staining for CD11b-positive cells (arrows) in the outer stripe of outer medulla in sham-operated (*B*), ischemic ARF + vehicle (*C*), and ischemic ARF+LEC (*D*) are demonstrated.

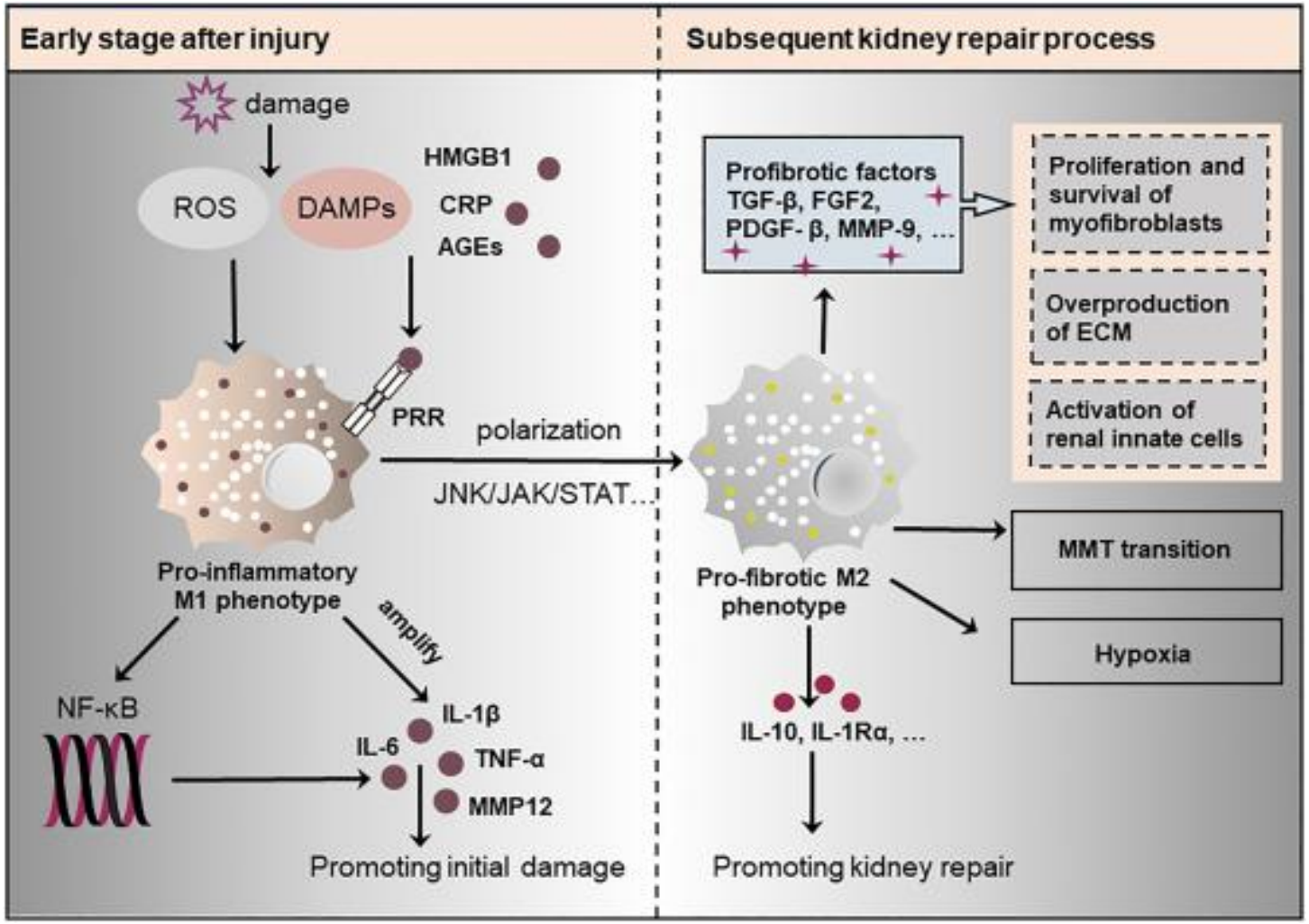
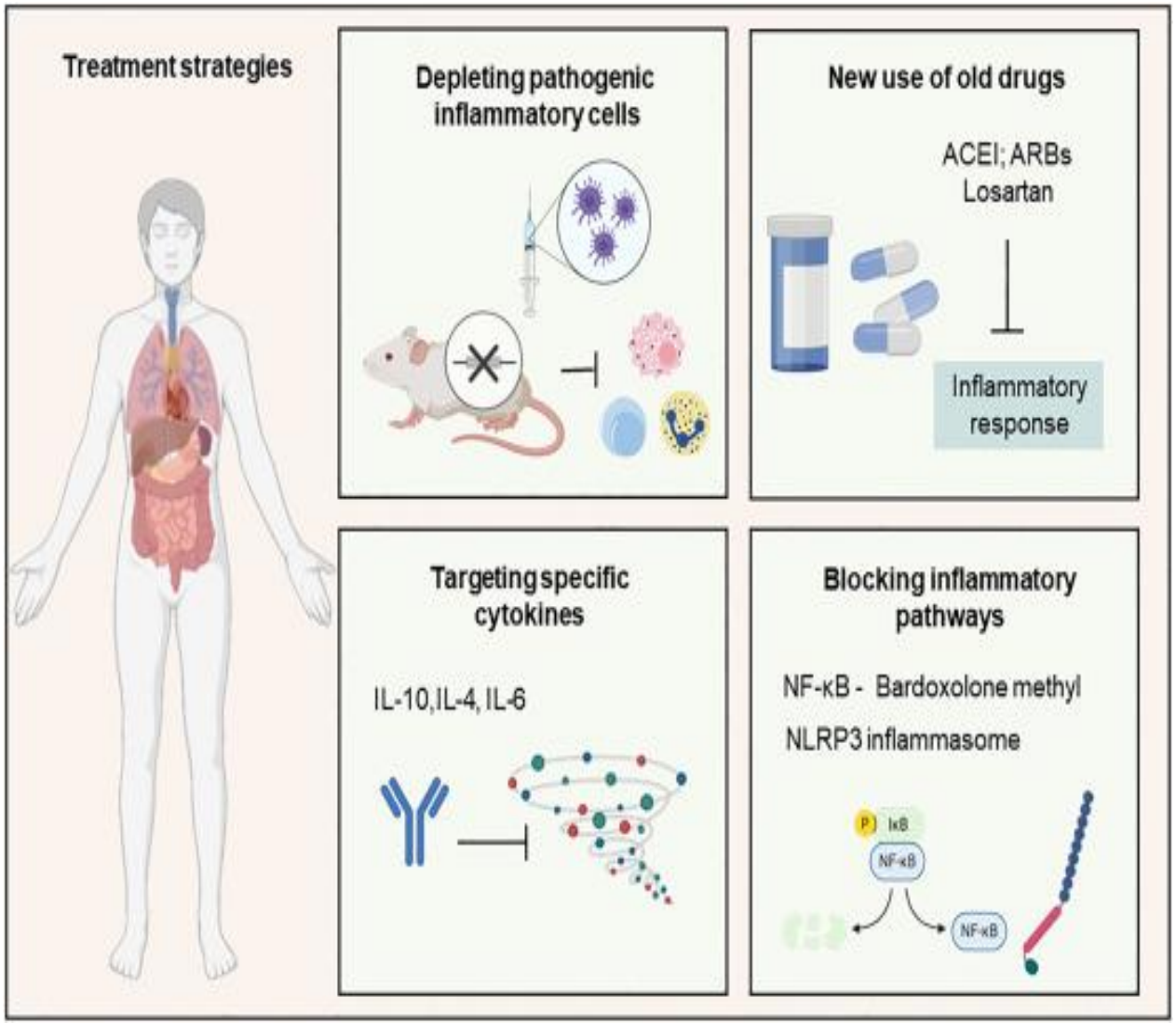


Fig. 1. Macrophages in kidney repair.
During kidney injury, damage-associated molecular patterns (DAMPs) are released from necrotic and damaged renal tubular cells. These DAMPs interact with pattern recognition receptors (PRRs) on macrophages, leading to macrophage activation. **In the early stage, activated macrophages are predominantly of the M1 phenotype and produce inflammatory cytokines to promote injury. However, during kidney repair, inflammatory M1 macrophages undergo a phenotypic switch to a non-inflammatory M2 phenotype, which produces pro-fibrotic factors such as TGF-β1, FGF-2, PDGF, and MMP-9 to activate fibroblasts. In addition, macrophages may directly contribute to the mesenchymal myofibroblast pool for renal fibrosis.** Macrophages may also damage glomerular and tubular capillaries to promote hypoxia in fibrotic tissues. Finally, M2 macrophages may produce and secrete repair-promoting cytokines such as IL-10 and IL-1Rα.

FU, Y. Pharmacology & Therapeutics, 2022

Versatile Macrophage Functions on Acute Kidney Injury and Its Outcomes

- **At the initial injury phase, M1 macrophages produce various cytokines** for robust inflammation.
- **During kidney repair, macrophages change their phenotype to M2, which produce pro-fibrotic factors** or may directly convert into myofibroblasts, leading to the development of interstitial fibrosis.



FU, Y. Pharmacology & Therapeutics, 2022

Targeting inflammation to improve kidney repair

- Infusion of **IL-10-transfected macrophages** improved renal function in rats with IRI.

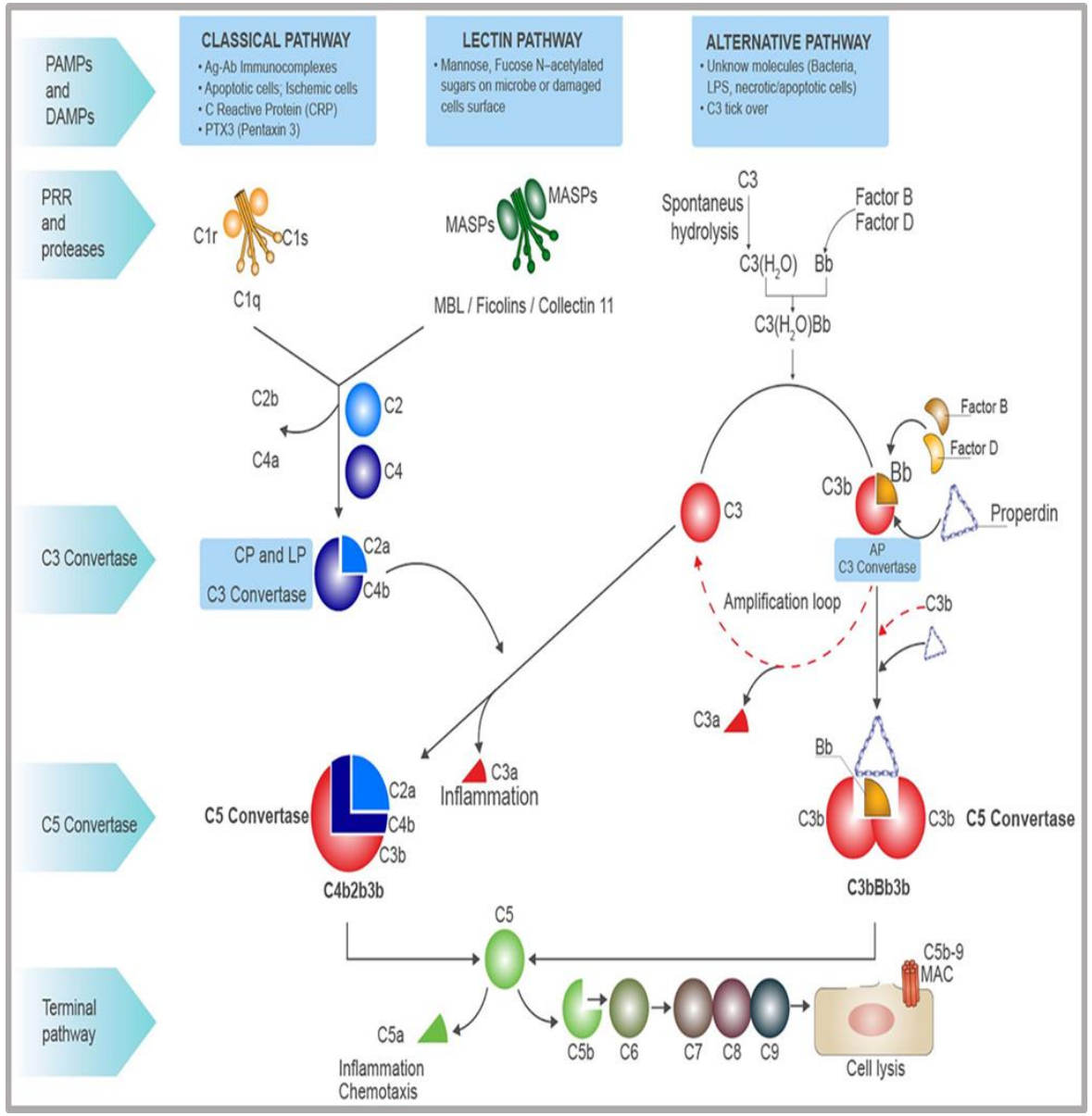
  Jung. Kidney Int 2012

- Impaired IL-18 processing protects **caspase1-deficient** mice from ischemic acute renal failure.

  Melnikov VY, J Clin Invest 2001

Kidney is the target of complement-mediated inflammation

Complement cascade is a vital component of innate immunity, uncontrolled complement activation is pathogenic



- Complement system includes **soluble proteins**, **cell surface receptors** and **regulatory proteins**.
- Complement proteins produced in **liver** and **other tissues (kidney**, etc)
- **Major biological roles**: host defense, clearance of cellular debris and immune modulation (bridges innate and adaptive immunity)
- The major goal of all three pathways:
  - **opsonization**, release of **proinflammatory anaphylatoxins (C3a and C5a)** and **assembly of the membrane attack complex (MAC)**.

Franzin R. Front. Immunol., 2020

Complement receptors: Ligands and functions

Receptor	Major ligands	Functions
CR1	C3b/C4b, C1q, MBL, ficolins	Immune adherence; immune complex clearance; regulation of C3b/C4b
CR2	iC3b, C3dg, C3d, IgE receptor, interferon alpha, DNA	B cell coreceptor; immune complex localization; EBV receptor; presents complement-opsonized antigens to T cells
CR3	iC3b, clotting factor X and up to 50 other ligands	Phagocytosis; neutrophil activation; apoptosis; cell activation
CR4	iC3b/fibrinogen/ICAMs	Phagocytosis; cell activation
C3aR	C3a	Cell type dependent; cell activation; histamine release
C4aR	C4a	Cell activation; endothelial cell permeability
C5aR1	C5a	Cell activation; chemotaxis; development and regeneration
C5aR2	C5a	Possible functions include nonsignaling decoy receptor; modulator of C5aR1; G-protein independent signaling
C1qR*	C1q*	Phagocytosis; a variety of C1q receptors have been identified with varied functions*
CRIg	C3b/iC3b	Phagocytosis; alternative pathway inhibitor

CR: complement receptor; MBL: mannose-binding lectin; EBV: Epstein-Barr virus; ICAMs: intercellular cell adhesion molecules; CRIg: complement receptor of the immunoglobulin superfamily.
* Ten putative C1q receptors with diverse structures have been proposed[1]. All bind C1q as well as other ligands triggering distinct cellular responses. However, these interactions do not lead to complement activation.

References:
1. *Bohlson SS, O'Conner SD, Hulsebus HJ, et al. Complement, C1q, and C1q-related molecules regulate macrophage polarization. Front Immunol 2014; 5:402.*

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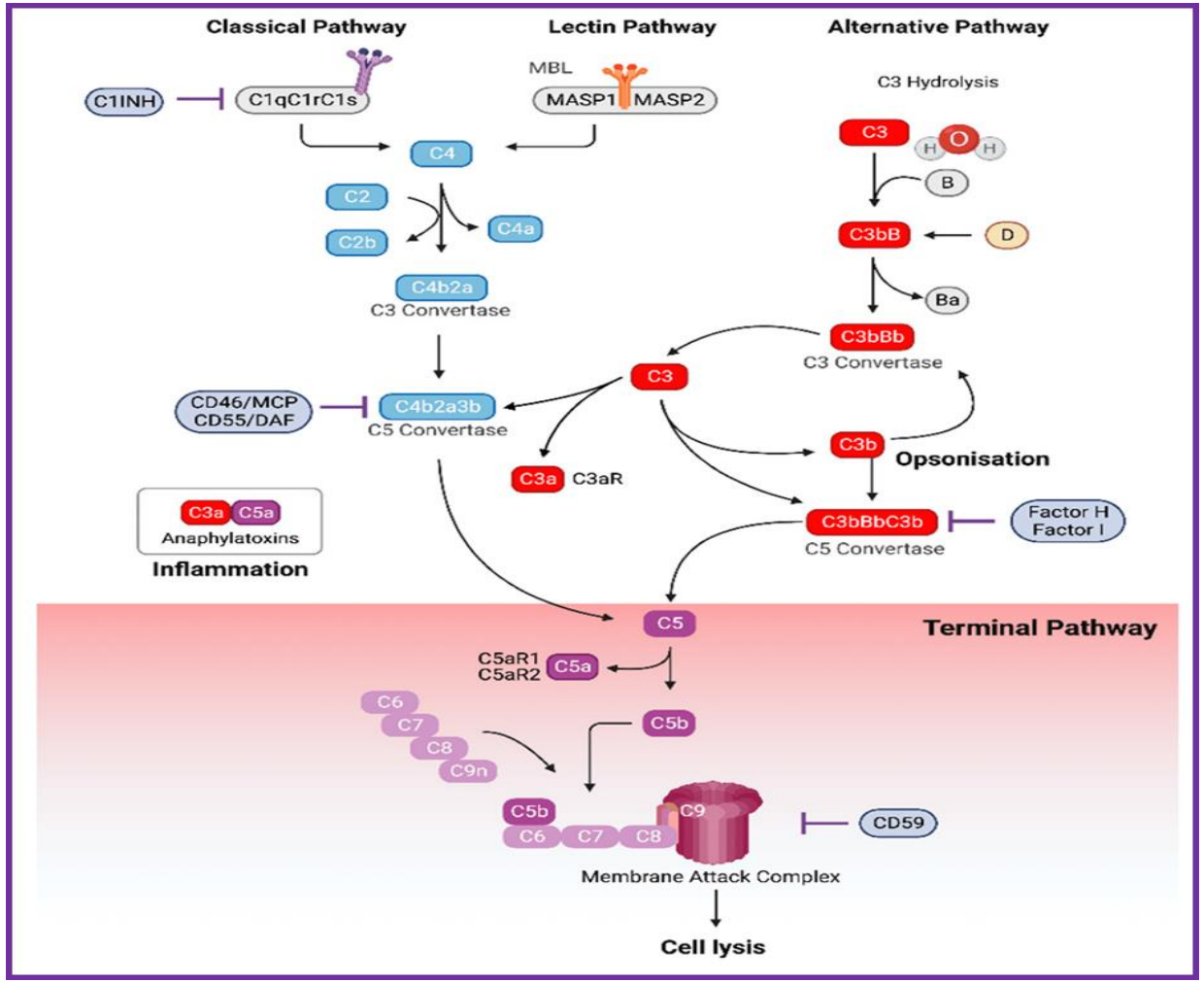
- **Receptors for complement activation fragments are expressed on many host cells**, including immune cells, endothelial cells, and epithelial cells.
- **Receptors for C3a and C5a are widely distributed** where they trigger the **local inflammatory response (innate immunity**) and also cell activation to prepare for the adaptive immune response.

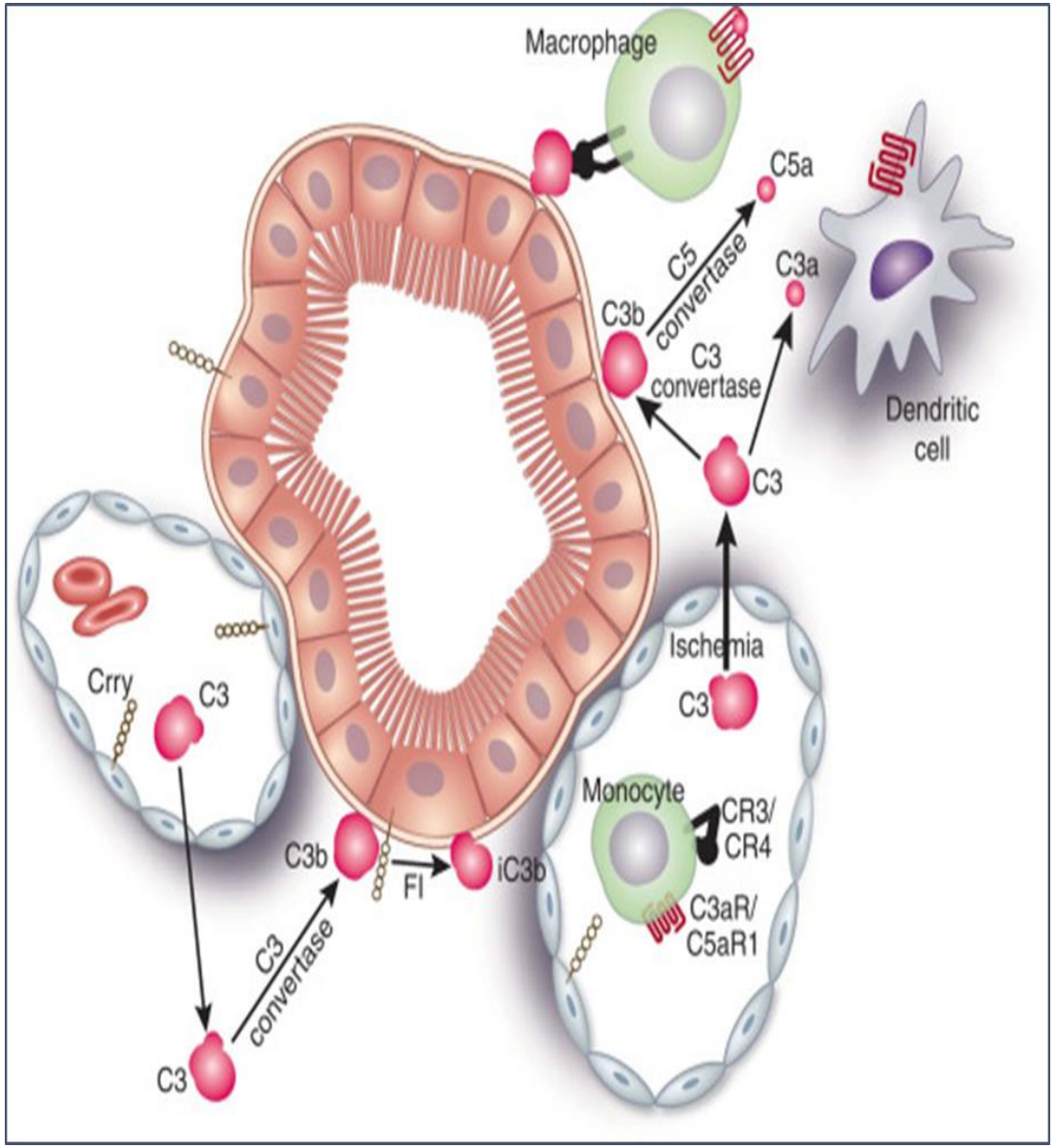
Defective complement regulation is associated with inflammatory diseases



Complement system is controlled by **regulatory proteins expressed on cell surface and in plasma:**

- **C1INH**: inhibits both classical and lectin pathways
- **Factor H**: alternative pathway regulator
- **Factor I**: inactivates C3b (**MCP/CD46** is co-factor Factor I)
- **CRIg** (complement receptor of Ig family) blocks C convertase, inhibits alternative pathway activation
- **DAF/CD55**: breakdown of C3 and C5 convertases

Zipfel PF. Nat Rev Immunol 2009



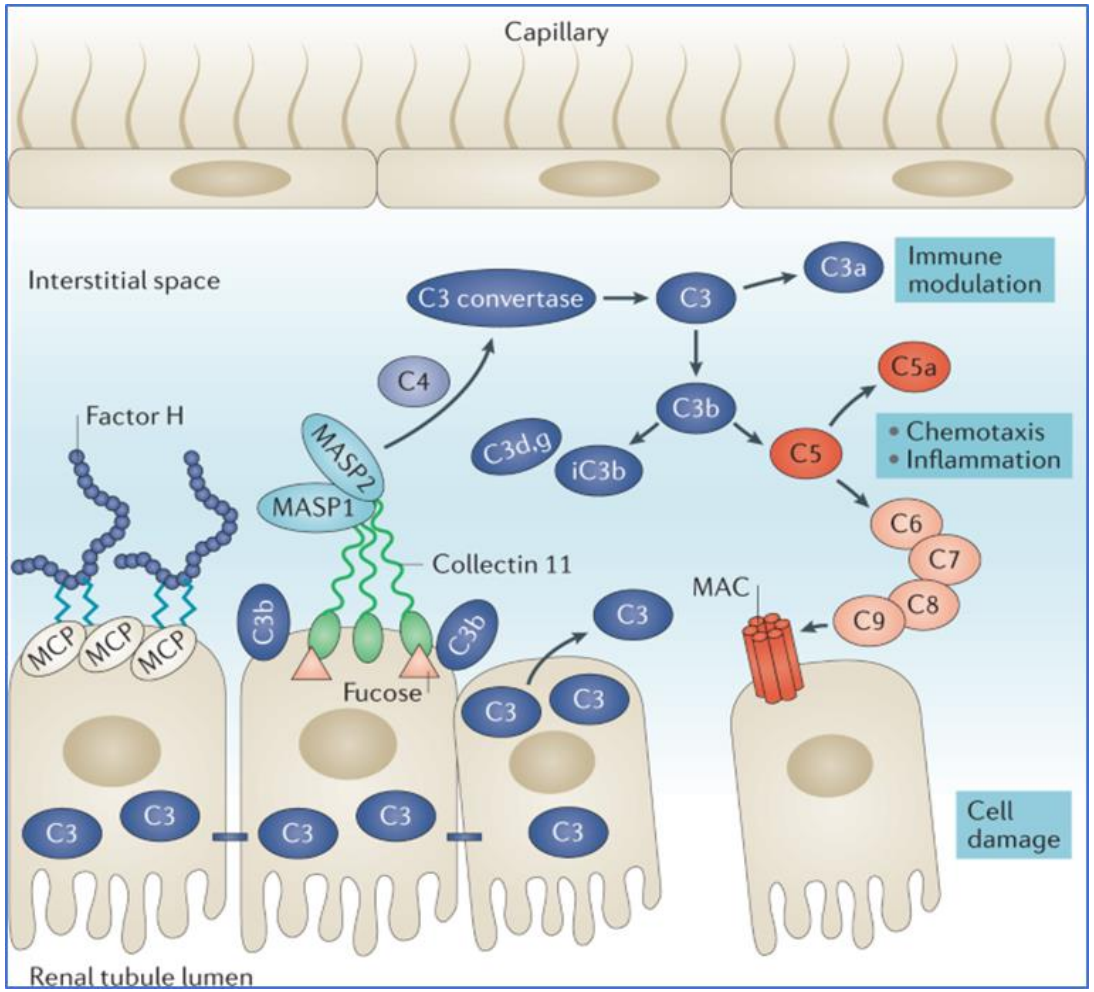
Brar JE, Quigg RJ. Kidney Int 2014

In AKI, complement activation primarily occurs within tubulointerstitium and peritubular capillaries

- Complement activating proteins concentrated in glomerular capilleries due to decreased filtration
- **Acidic environment** and **ammonia synthesis promote alternative pathway activation, and** may also play role in the progression of CKD.

  Nath KA, et al. J Clin Invest 1985
- **Inflammatory environment** is effective initiator of alternative pathway and excessive C3 production.

Complement Activation Pathways in ischemic AKI



Ischaemic stress induces the upregulation of fucose and the basolateral secretion of collectin 11 in renal tubule cells[48,49]. In the presence of locally produced complement components or upon reperfusion, mannan-binding lectin serine protease 1 (MASP1) and MASP2 bind to collectin 11 and initiate the lectin pathway of complement activation leading to the release of anaphylatoxins and a destructive inflammatory response that may ultimately result in the formation of the membrane attack complex (MAC) and cell damage. This response is further fuelled by C3, which is locally synthesized in the renal tubular cells in response to ischaemia[51], and by the **ischaemia-induced loss of complement regulators such as membrane cofactor protein (MCP; also known as CD46) and factor H, which facilitates complement activation and deposition, cell death and acute kidney injury**.

Biglarnia AR. Nature Reviews Nephrology, 2018

Important role of alternative pathway: (C3 breakdown increased & loss of regulatory proteins)

- In contrast to C4-/- mice, **mice lacking C3 (C3-/-) are protected from IRI.**

  Zhou Wet al . J Clin Invest 2000

- **Factor B -/-** mice **have improved protection from IR Injury and sepsis-AKI**.

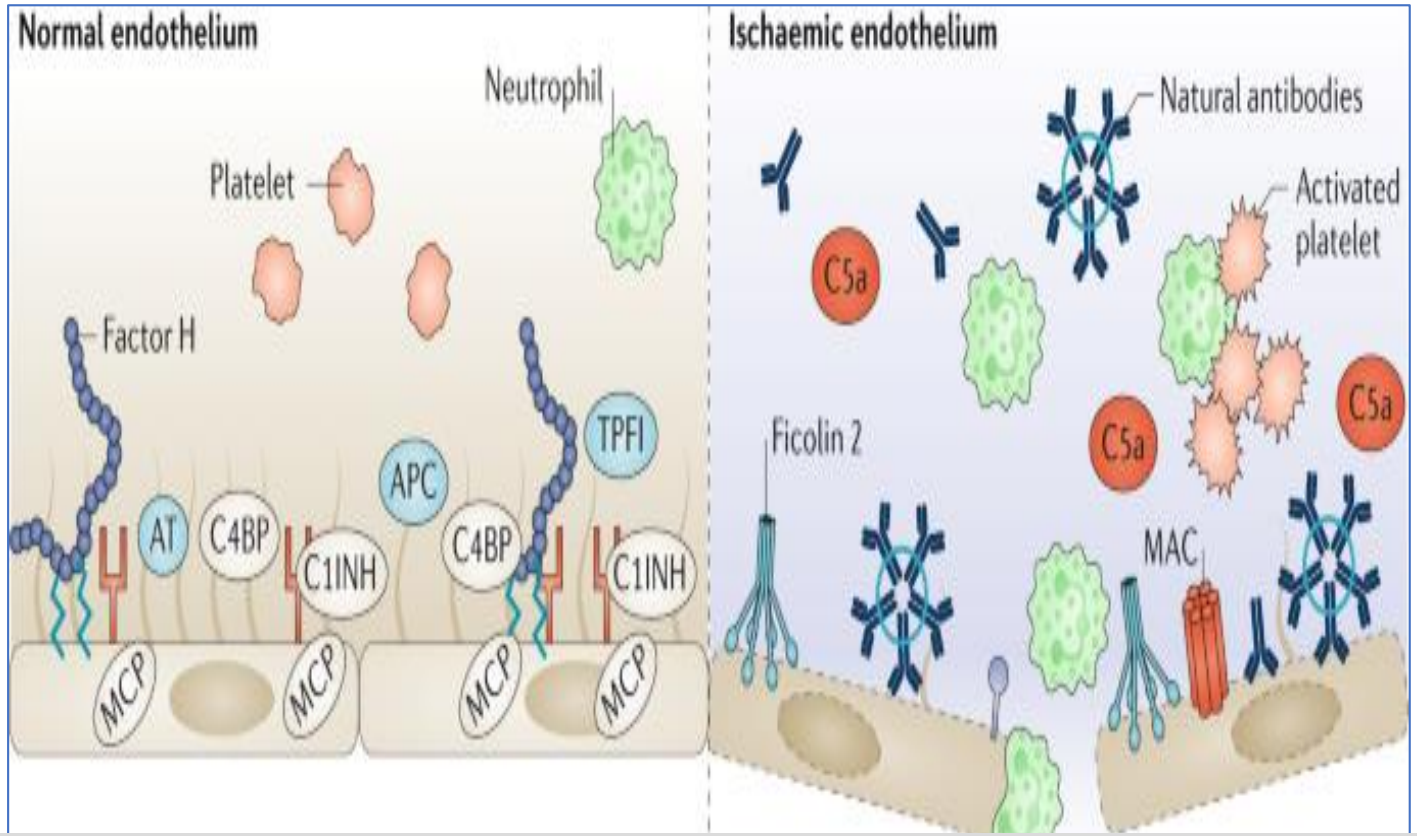
  Thurman JM. J Immunol 2003
  Zou L. J Immunol 2013

- **C3d deposits were demonstrated on RTE cells in human AKI biopsies.**

  Thurman JM. Kidney Int 2005

- Collectin 1 (Cl-11) released from ischemic RTE cells and binds L Fucose expressed on cell surfaces.
- **Lectin pathway activation further amplified through alternative pathway via cleavage of C3**.

  Farrar Ca. J Clin Invest 2016



The normoxic endothelium is protected by the glycocalyx, which has an affinity for and binds the fluid-phase regulators of the complement system C1 inhibitor (C1INH), factor H and C4b-binding protein (C4BP) as well as the coagulation inhibitors antithrombin (AT), tissue factor pathway inhibitor (TFPI) and activated protein C (APC). The endothelium also expresses membrane cofactor protein (MCP; also known as CD46) which inhibits C3 and C5 convertases. The combined effect of these regulators means that the normoxic endothelium is anti-inflammatory and antithrombotic. **During ischaemia and reperfusion, the endothelium becomes activated by cytokines and/or complement activation products such as the membrane attack complex (MAC), resulting in the breakdown of the glycocalyx and subsequent loss of regulators of the complement and coagulation systems**. In this setting complement activation can be driven by molecules such as ficolin 2 and natural antibodies, resulting in **the formation of complexes between activated platelets and polymorphonuclear leukocytes (PMNs) such as neutrophils. Activated PMNs bind to the endothelial cells and migrate through the endothelium into the parenchyma, thereby aggravating the inflammatory response.**

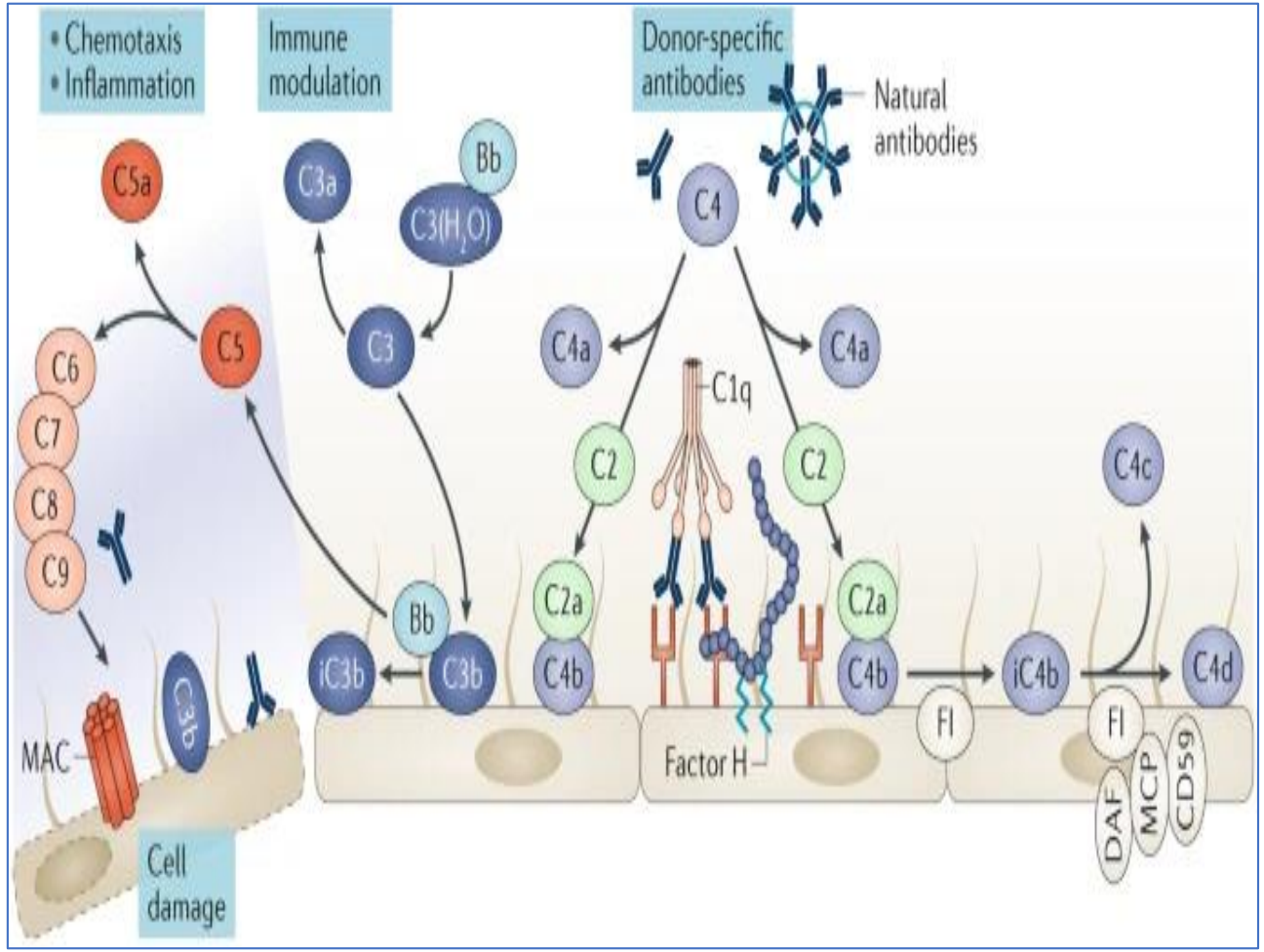
Biglarnia AR et al, Nature Rev Nephrol 2018

- Healthy endothelial cells control complement activation on cell surface effectively by regulatory proteins.
- **During IR there is breakdown of endothelial glycocalyx and loss of regulatory proteins**

inflammatory responses and coagulation increased

- C3-, C5-, C6- deficient transgenic mice are protected againts IR injury.

Thurman JM. J Immunol 2003



During antibody-mediated rejection, natural antibodies, for example antibodies against blood group antigens or donor-specific anti-HLA antibodies bind to their targets on the endothelial cells of the graft and trigger activation of the classical pathway of complement via assembly of the C3 convertase C4bC2a on the endothelium. **This activation is further amplified by the alternative pathway via formation of the C3 convertase C3bBb, which ultimately leads to formation of the membrane attack complex (MAC)**. Complement activation is counteracted by the cell-bound regulators complement-decay accelerating factor (DAF; also known as CD55), membrane cofactor protein (MCP; also known as CD46) and CD59 glycoprotein, as well as by factor H, which is present in the blood and on the glomerular basement membrane and inhibits the activation of the alternative pathway. FI, factor I.

Biglarnia AR et al, Nature Rev Nephrol 2018

- **Donor-specific anti-HLA antibodies** bind to their targets on the endothelial cells of the graft and trigger **activation of the classical pathway of complement.**
- **This activation is further amplified by the alternative pathway** which ultimately leads to **formation of the membrane attack complex (MAC).**

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Basic Immunology and Pathology

Treatment with an Inhibitory Monoclonal Antibody to Mouse Factor B Protects Mice from Induction of Apoptosis and Renal Ischemia/Reperfusion Injury

Joshua M. Thurman, Pamela A. Royer, Danica Ljubanovic, Belda Dursun, Amanda M. Lenderink, Charles L. Edelstein and V. Michael Holers

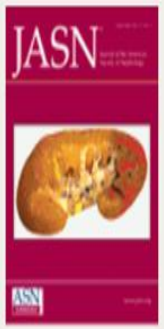
JASN March 2006, 17 (3) 707-715; DOI: https://doi.org/10.1681/ASN.2005070698

Article | Figures & Data Supps | Info & Metrics | View PDF

Abstract

Complement activation in the kidney after ischemia/reperfusion (I/R) seems to occur primarily *via* the alternative complement pathway. The ability of an inhibitory mAb to mouse factor B, a necessary component of the alternative pathway, to protect mice from ischemic acute renal failure was tested. Treatment with the mAb prevented the deposition of C3b on the tubular epithelium and the generation of systemic C3a after renal I/R. Treated mice had significantly lower increases in serum urea nitrogen and developed significantly less morphologic injury of the kidney after I/R. For gaining insight into potential mechanisms of protection, the activity of caspases within the kidney also was measured, and it was found that caspases-2, -3, and -9 increased in a complement-dependent manner after renal I/R. Apoptotic cells were detected by terminal deoxynucleotidyl transferase catalyzed labeling of DNA fragments, and mice in which the alternative pathway was inhibited demonstrated significantly less apoptosis than control mice. Thus, use of an inhibitory mAb to mouse factor B effectively prevented activation of complement in the kidney after I/R and protected the mice from necrotic and apoptotic injury of the tubules.

In this issue



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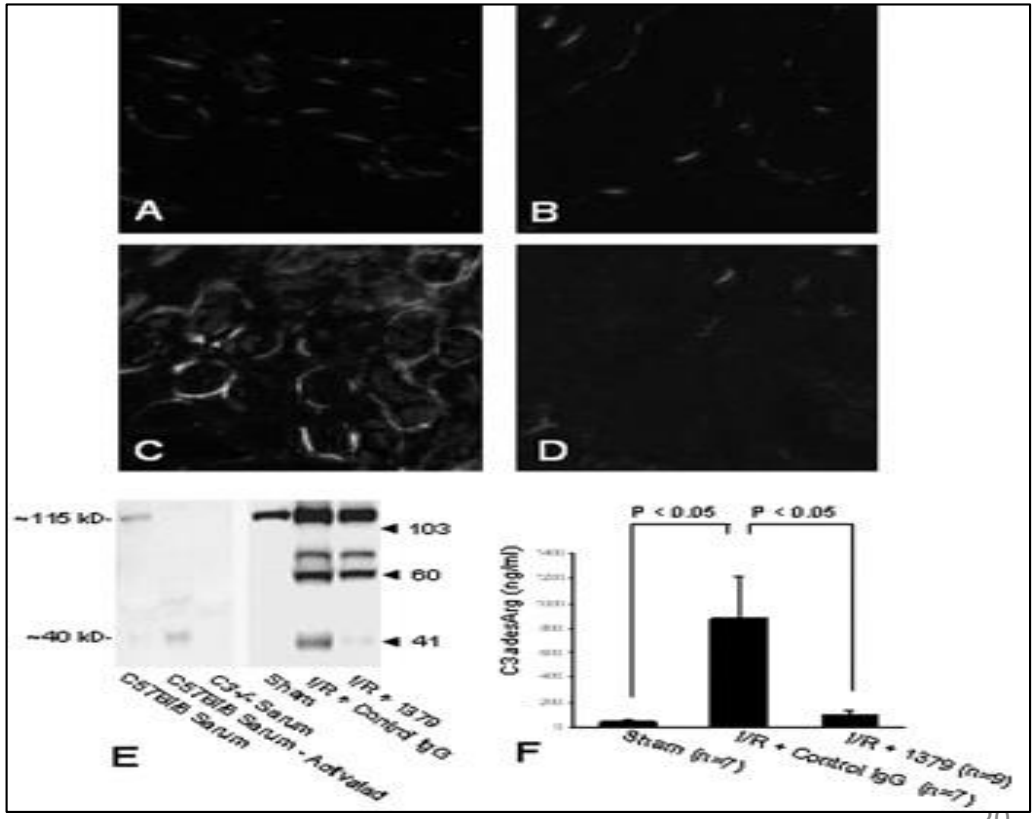
Alternative pathway blokage (mAb 1379 to mouse factor B, necessary component of alternative pathway) prevented generation of systemic C3a and deposition of C3b on tubular epithelium after IR injury , **provided functionally and morphologically less AKI** (caspase-2,3-9 mediated tubular apoptosis and necrosis prevented).



Complement activation in the tubulointerstitium: AKI, CKD, and in between

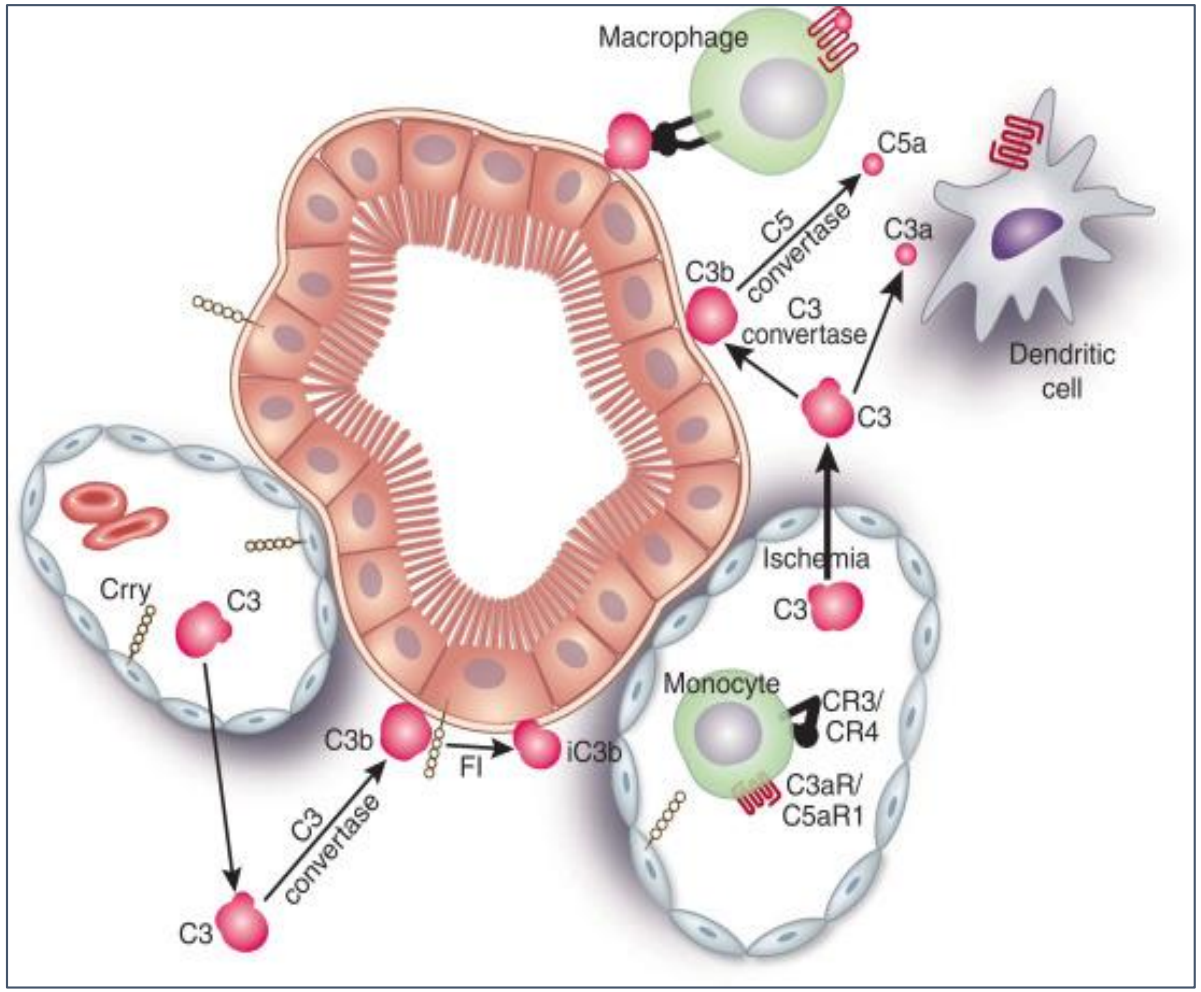


Figure 1. **Schematic view of the renal cortical tubulointerstitium.** Crry is present in the basolateral aspects of tubular cells and the peritubular endothelium. C3 is abundantly present in plasma and accesses the wide interstitial space. There, activation of the alternative pathway, in part or wholly by local ammonia, can generate C3b, which can bind to the basolateral aspects of tubules. Under normal conditions, this is inactivated to iC3b by factor I (FI) with a suitable cofactor such as Crry or factor H. Following ischemia–reperfusion, there is likely greater access of C3 and C5 to the interstitial space along with altered polarization of Crry. In this setting, the balance is tipped toward productive C3 and C5 activation, with generation of active complement fragments. These can lead to inflammation with extrinsic and intrinsic mononuclear cells (macrophages and dendritic cells), and direct cellular injury.

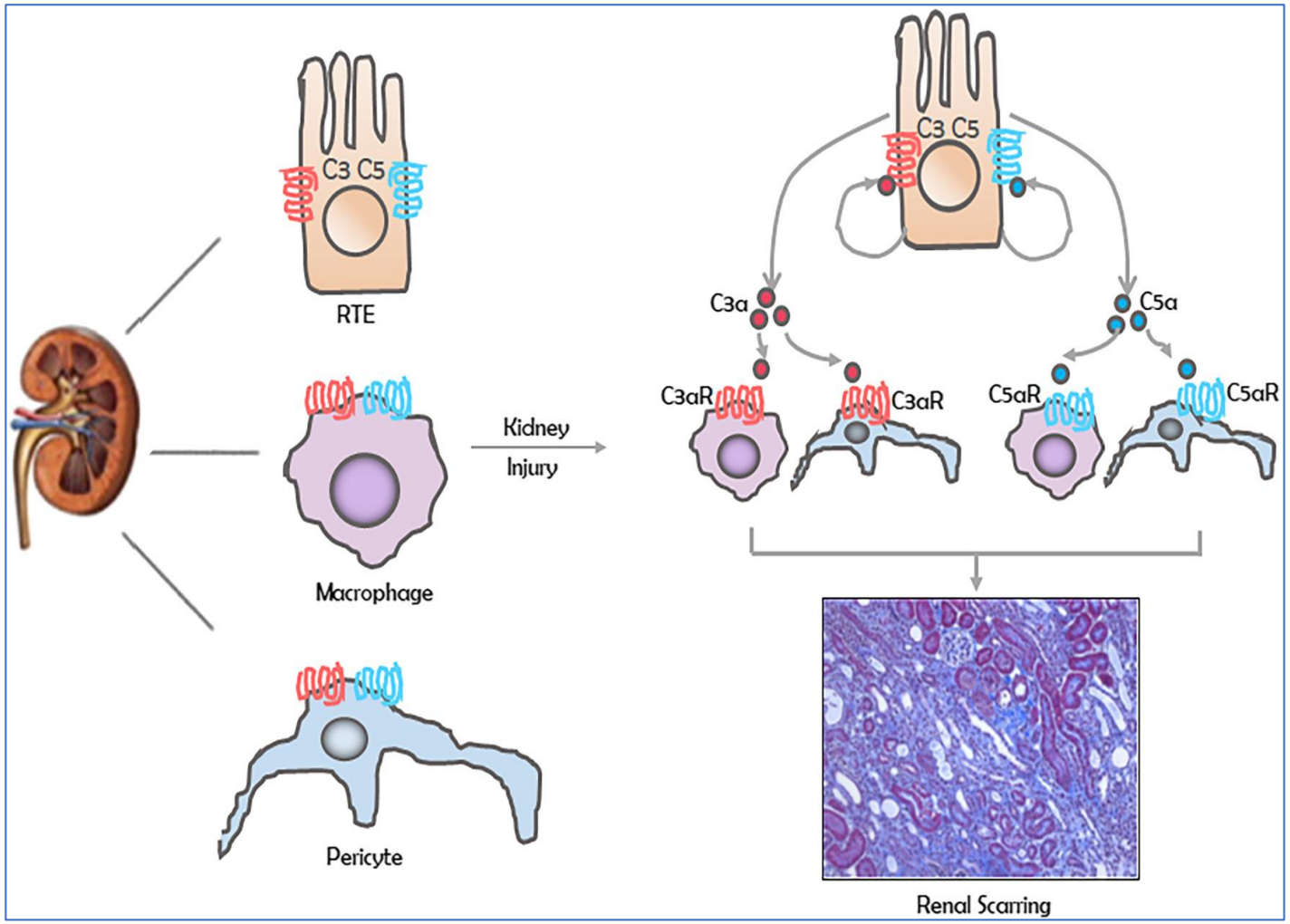
Brar JE, Quigg RJ. Kidney Int 2014

- **Rodent complement regulator Crry** (similar to MCP in humans) is present in renal endothelial and epithelial cells.
- **Normal polarization of Crry is lost in ischemia** leading to **unrestricted alternative pathway activation.**

- Crry-/- mice are more susceptible to IR than WT mice.
  Thurman JM, J Clin Invest 2006
- Crry-/- C3-/- kidneys transplanted into WT mice:
  - acute activation of plasma derived C3 led to AKI and then CKD within weeks. *Bao L. JASN 2007*

- **Complement activation results in C3aR activation on monocytes/macrophages** and **proliferation of resident dendritic cells**.
  Chaves LD. PlosOne 2014

Role of intracellular complement activation in kidney fibrosis

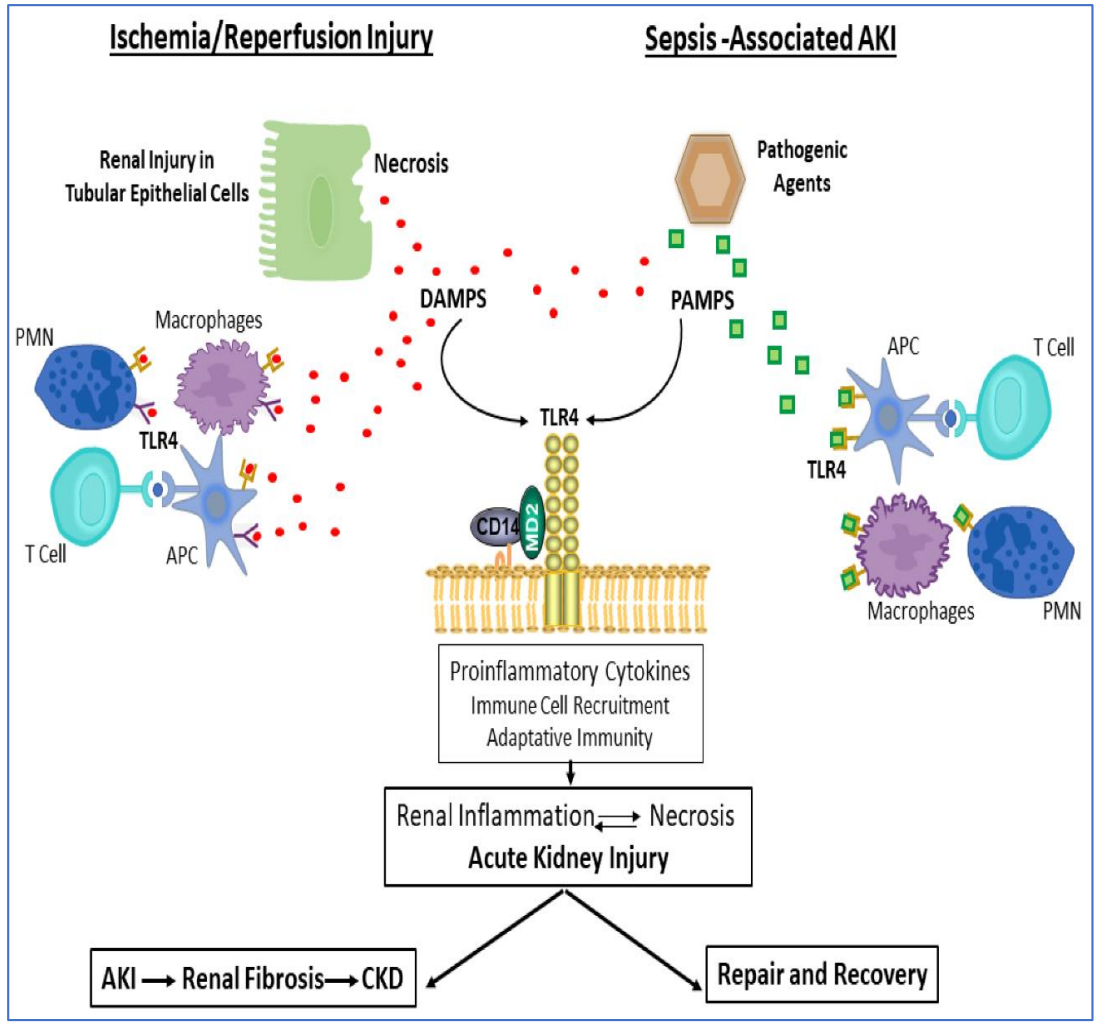


Kidney injury enhances production of complement components C3 and C5 by renal tubular epithelial cell (RTE). Processed fragments of these C3a and C5a ligand their cognate receptors C3a (C3ar1) and $C5a_1$ (C5ar1) on tissue macrophages and pericytes to drive renal fibrosis

Portilla D, Xavier S. Br J Pharmacol 2021

- Crry-/- C3-/- kidneys tranplanted into hosts lacking C3ar1 and C5a1r led to reduced TI inflammation and fibrosis
  Bao L. Kidney Int 2011
- Blockade of C3ar1 and/or C5ar1 protects against IRI and immune cell infiltration
  Peng Q. JASN 2012
- **Current data indicate both renal resident and infiltrating immune cells** contribute to IR injury.

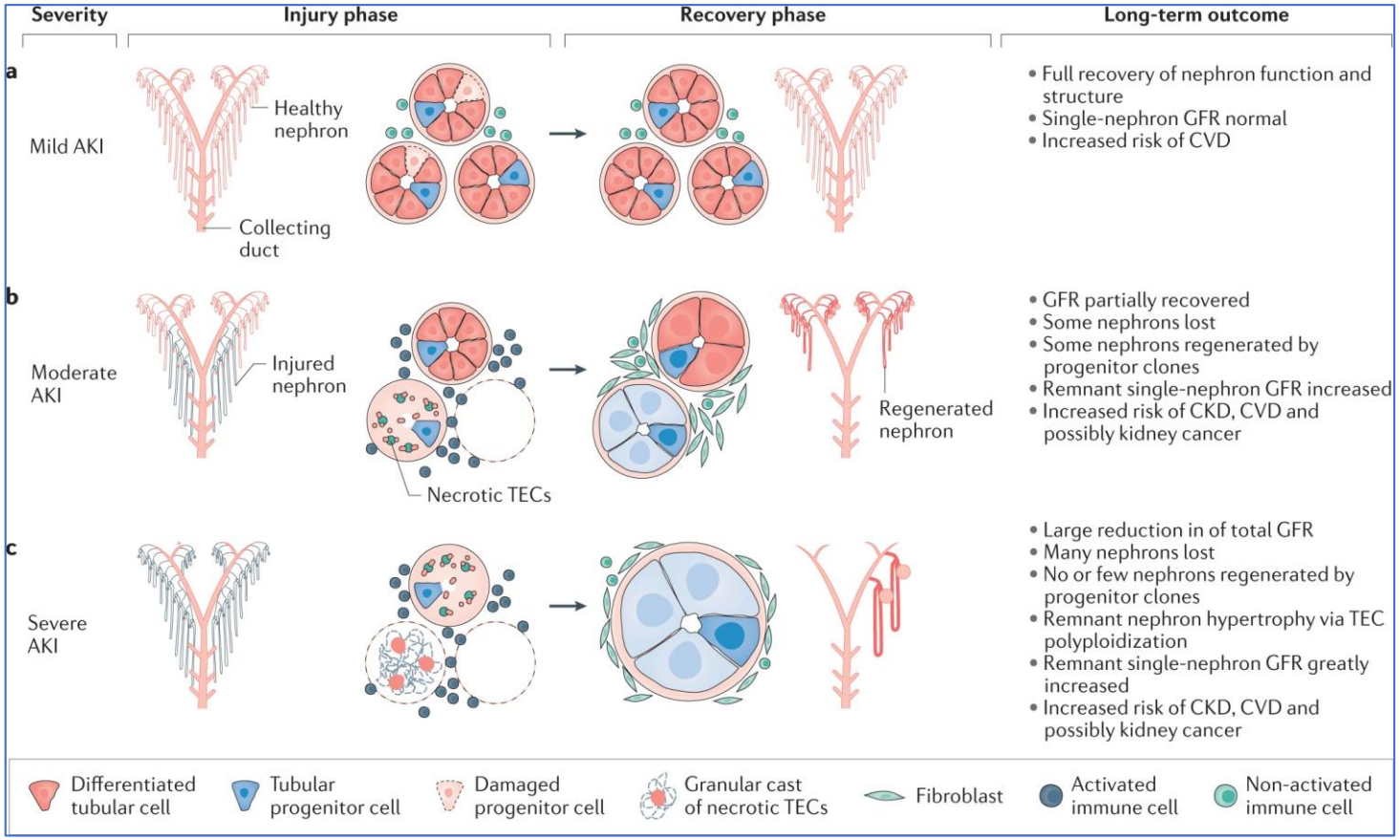
Toll-like receptors (TLRs) are a family of pattern **recognition receptors (PRRs)** in the first line defense system widely **expressed on leukocytes and TEC**



Bi-directional cross talk between TLRs, complement and cytokines

- ❑ TLR induce expression of complement components
- ❑ Complement receptors regulate TLR-dependent responses.
- ❑ TLR prime cells to undergo **pyroptosis** (programmed cell death), **cellular lysis and proinflammatory cytokine release** (pro-IL-1β, IFN-α/β and NF- κB dependent cytokines)
- ❑ Proinflammatory cytokine events transmitted systematically and may lead to **extended inflammation and distant organ dysfunctions.**

Valles PG. Int J Mol Sci 2023



a | Mild acute kidney injury (AKI), defined by a transient decline in urinary output or excretory function, involves no or minimal kidney cell necrosis or loss. Precedent and subsequent nephron numbers remain identical and no persistent adaptive cellular responses are necessary. In the long term, the risk of cardiovascular disease (CVD) is somewhat increased, which may also depend on the underlying cause of AKI. **b** | Whenever AKI is associated with kidney cell or tubule necrosis, the affected cells are irreversibly lost during the phase of acute necroinflammation, as indicated by activated immune cells in the interstitial compartment. Renal progenitor cells are more resistant to death and their clonal expansion may facilitate the structural and functional recovery of some injured nephrons. Nephrons in which injured segments do not recover undergo atrophy, are irreversibly lost and are replaced by fibrous tissue that stabilizes the structural integrity of remnant nephrons. Resulting hyperfiltration requires an increase of the functional capacity of remnant nephrons achieved through an increase in their dimensions, with tubular epithelial cells (TECs) undergoing polyploidization, indicated by an increased size of cytoplasm and cell nuclei. Depending on the number of remnant nephrons, their capacity for adaption (kidney reserve), and filtration load (dependent on body weight, fluid intake, diet and others), glomerular filtration rate (GFR) can return to baseline. This status already qualifies as CKD, even if GFR returns to baseline. The adaptive changes of CKD imply a higher risk of CVD and possibly kidney cancer, and the irreversible loss of nephrons reduces kidney lifespan. **c** | When severe AKI involves extensive tubule necrosis, the consequences on nephron number are substantial. Tubule recovery occurs only in those nephrons with surviving progenitor cells. Adaptation to filtration and metabolic demands results in large increases in the dimensions of the few surviving nephrons (megalonephrons). Such adaptations frequently exceed the adaptive capacity of podocytes, leading to secondary focal segmental glomerulosclerosis and subsequent loss of the remnant nephrons (that is, progressive CKD). Cellular adaptation-related polyploidization and senescence, as well as nephron loss-related scarring, drives interstitial fibrosis and progressive kidney atrophy. These adaptive changes strongly increase the risk of CVD and possibly kidney cancer. Kidney lifespan is drastically reduced and some patients remain on kidney replacement therapy

- **After AKI, the kidney has a remarkable capacity for repair**, involving dedifferentiation, proliferation, and redifferentiation of tubular epithelial cells.
- However, **when injury is severe or persistent, the repair is incomplete or maladaptive and may lead to chronic kidney disease (CKD)**

Kellum JA et al. Nature Reviews Disease Primers 2021

Maladaptive Kidney Repair

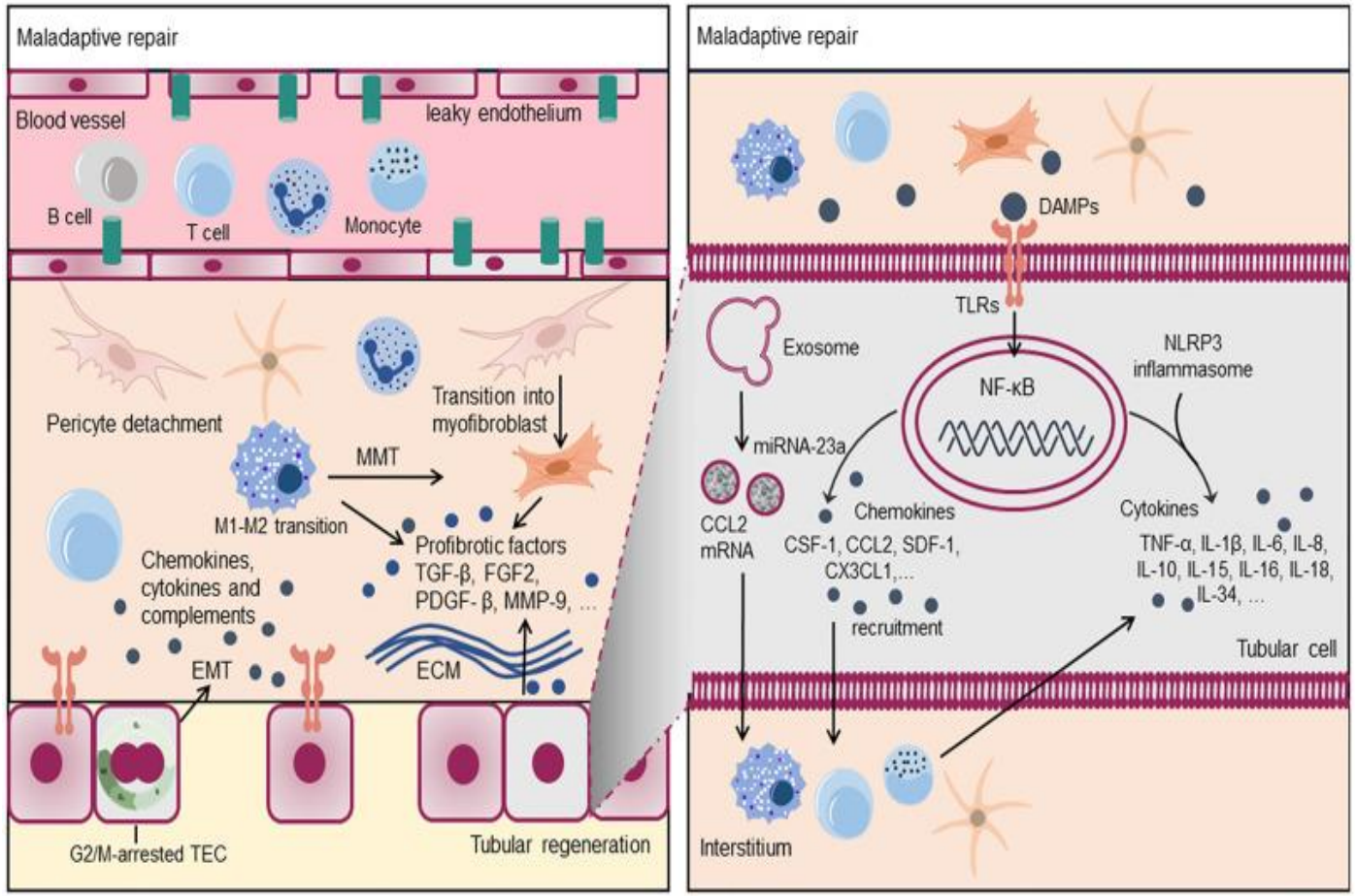
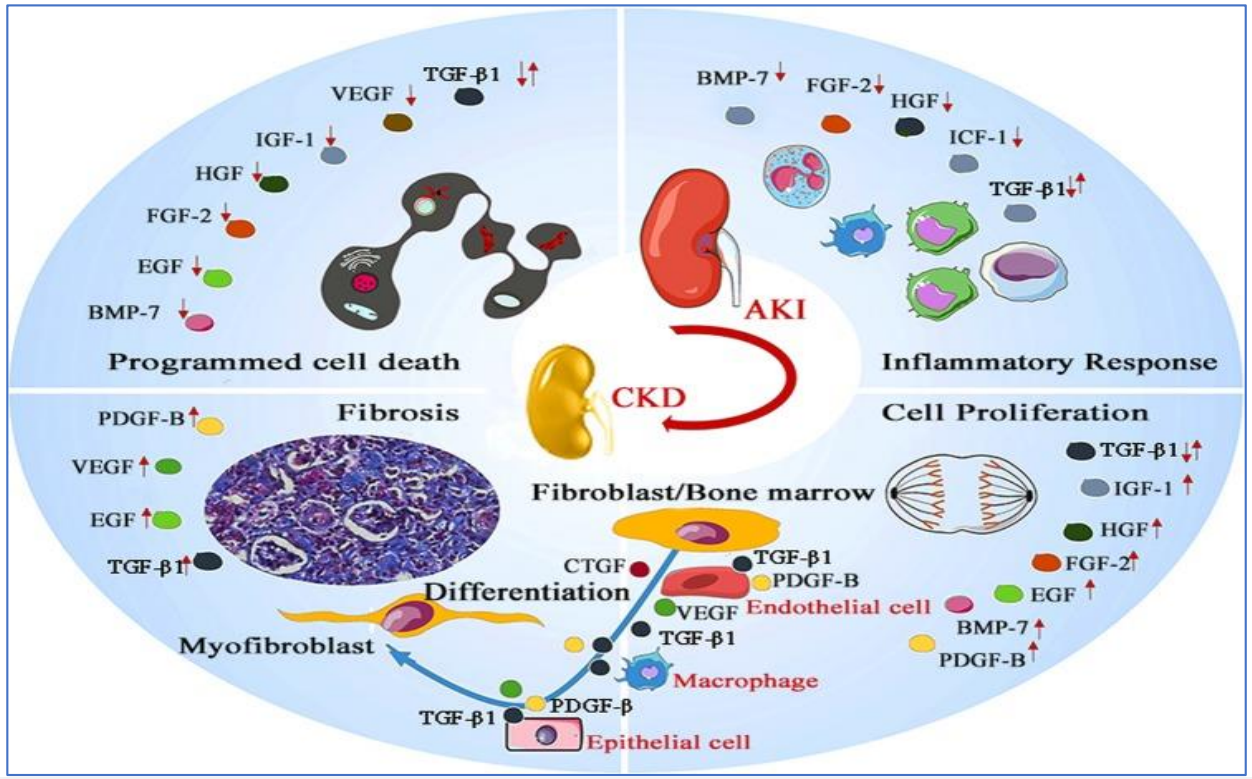


Fig. 2. Immune cells and renal intrinsic cells in maladaptive kidney repair.
Immune cells, including T cells, B cells, neutrophils, monocytes, etc., are normally localized in renal microvessels. During kidney injury, these immune cells adhere to activated endothelial cells as a result of vascular damage and the chemoattractant activity of chemokines. Some immune cells migrate into the renal interstitium and undergo phenotypic transitions, such as the M1-M2 transition of macrophages. On the one hand, the immune cells may secrete pro-fibrotic growth factors or transform into myofibroblasts. On the other hand, they secrete chemokines, cytokines, and complements, thereby aggravating the cycle of renal inflammation. Meanwhile, tubular regeneration occurs during this process. Specifically, receptors on TECs, such as TLRs, receive paracrine signals such as DAMPs, activating the transcription of intracellular inflammatory factors or chemokines and further inducing inflammatory cells to enter the renal interstitium. Exosomes produced by TECs can also mediate intercellular crosstalk and exacerbate inflammation in maladaptive kidney repair.

- Maladaptive kidney repair involves **multiple cell types and multifactorial processes,** of which **inflammation is a key component.**
- In the process of inflammation, there is a **bidirectional interplay between kidney parenchymal cells and the immune system.**

FU, Y. Pharmacology & Therapeutics, 2022

Effect of Growth Factors on AKI and AKI-CKD Transition



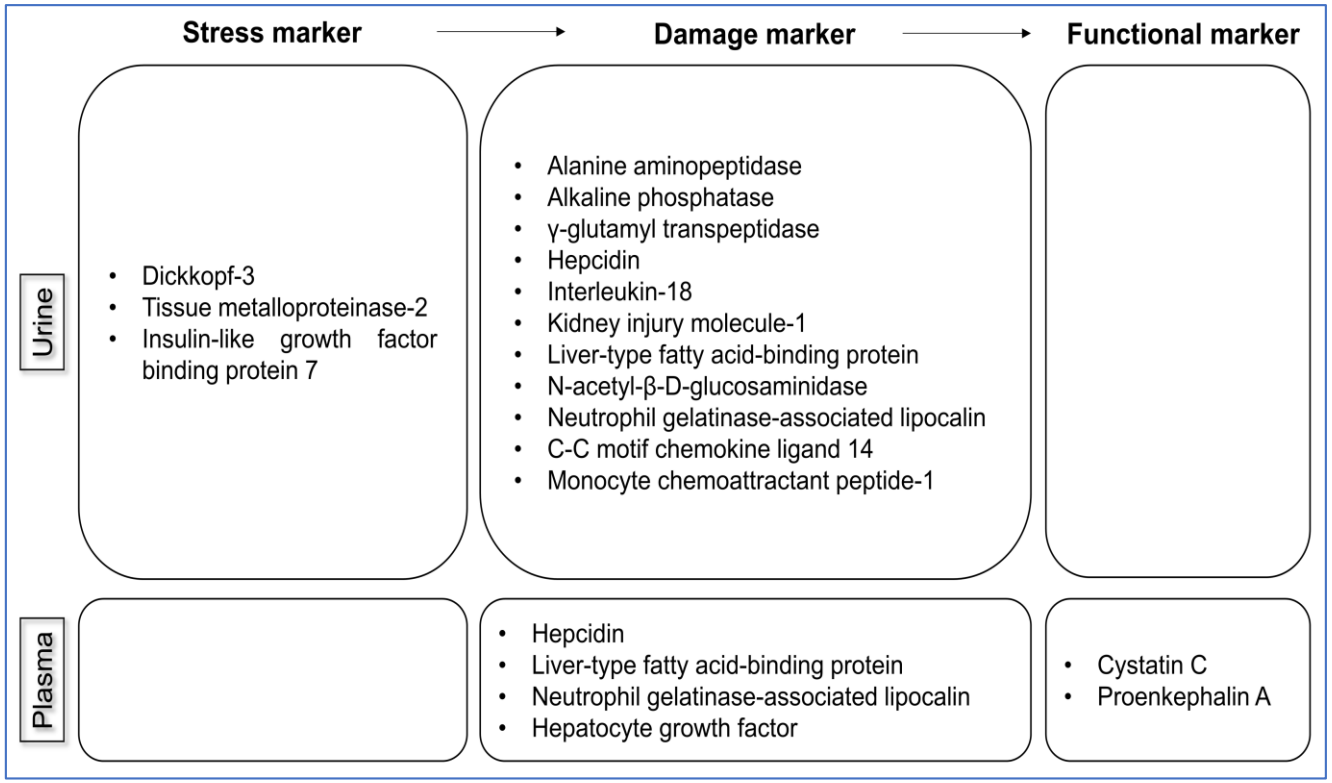
Effect of growth factors on AKI and AKI-CKD progression. Many growth factors, such as BMP-7, EGF, FGF-2, HGF, IGF-1, VEGF, and TGF-β1, are involved in the programmed cell death of endothelial or epithelial cells in the acute injury phase. BMP-7, FGF-2, HGF, TGF-β1, and IGF-1 participate in the regulation of the inflammatory microenvironment that is responsible for cytokine production and immune cell recruitment. TGF-β1 is a double-edged growth factor. In addition, TGF-β1 exerts anti-inflammatory effects, and TGF-β1 overproduction leads to acute tubular injury. After injured epithelial cells fail to regenerate through differentiation, fibrosis is induced as a self-limiting repair process to limit damage. In this stage, overproduction of growth factors such as TGF-β1, PDGF, and FGF induces fibroblast/pericyte proliferation, transdifferentiation of tubular epithelial cells, endothelial cells, and macrophages, and extracellular matrix production, leading to CKD. Concurrently, abnormal synthesis of PDGF-B, VEGF, EGF, and TGF-β1 has a negative impact on endothelial integrity and causes capillary rarefaction, accelerating renal fibrosis.

- **Many growth factors (BMP-7, EGF, FGF-2, HGF, IGF-1, VEGF, and TGF-β1)** involved in the **acute injury phase, inflammatory response (cytokine production and immune cell recruitment) and programmed cell death**

- **After injured epithelial cells fail to regenerate through differentiation, fibrosis is induced leading to CKD**
  - overproduction of growth factors (TGF-β1, PDGF, and FGF)stimulates fibroblast/pericyte proliferation, transdifferentiation of tubular epithelial cells, endothelial cells, macrophages, and ECM production.

Signal Transduct Target Ther. 2020

AKI biomarkers from the Acute Disease Quality Initiative Consensus Conference-(ADQI-23)



The urinary markers tissue metalloproteinase-2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP7):

- ❑ recently discovered inducers of G1 cell-cycle arrest and are key stress biomarkers of AKI,
- ❑ considered superior to known damage biomarkers such as kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL)
- ❑ **urinary biomarker [TIMP-2] × [IGFBP7] approved in the United States and Europe**

Three types of biomarkers exist based on the recommendations on AKI biomarkers from the Acute Disease Quality Initiative Consensus Conference (Figure 1). Stress markers reflect cell stress, which may resolve or become aggravated [7]. A damage marker indicates structural damage that may or may not result in a reduction in renal function [7]. Functional markers correlate with alterations in glomerular filtration [7]. Considering these biomarkers together can offer a precise approach beyond measuring the SCr level or UO alone, and may suggest the most accurate diagnostic and therapeutic methods.

Table 1 Complement factors with potential use as AKI biomarkers

Biomarker	Population/disease state	Significant findings
Urine Ba	Adults post-cardiac surgery [71]	↑ Urine Ba = ↑AKI severity
	Critically ill children [74]	↑ Urine Ba = ↑AKI severity
	FSGS [82]	↑ Urine Ba at diagnosis ctc
Urine Bb	ANCA-associated vasculitis [83]	↑ Urine Bb in active disease vs. disease remission
	FSGS [84]	↑ Urine Bb ctc
Plasma Ba	Adults with TA-TMA [85]	↑ Plasma Ba → 2 weeks later = TA-TMA diagnosis
Plasma Ba	FSGS [82]	↑ Plasma Ba at diagnosis ctc
Plasma Bb	Adults with primary membranous nephropathy [84]	↑ Plasma Bb compared to control
Plasma Bb	FSGS [82]	↑ Plasma Bb = more severe disease
Urine C3	Critically ill adults with sepsis [86]	Urine C3a/C3 ratio is an inverse acute phase reactant
Urine C3a	FSGS [84, 87]	↑ Urine C3a ctc Urine C3a correlated with renal dysfunction, proteinuria, and interstitial fibrosis
Urine C3a	ANCA-associated vasculitis [83]	↑ Urine C3a in active disease vs. disease remission
Urine C3b	FSGS [88]	↑ Urine C3b ctc
Urine C3d	Lupus nephritis (LN) [89]	↑ Urine C3d elevated in active LN compared to inactive or chronic LN
	Tubulo-interstitial nephritis [90]	↑ Urine C3d ctc
Plasma C3a	Critically ill children [74]	↑ Plasma C3a = ↑AKI severity
	Adults with primary membranous nephropathy [84]	↑ Plasma C3a compared to control
	FSGS [84, 87]	Plasma C3a correlated with renal dysfunction, proteinuria, and interstitial fibrosis
Plasma C4a	Critically ill children [74]	↑ Plasma C4a = MAKE30 outcomes
Urine C4a	FSGS [82]	↑ Urine C4a at diagnosis ctc
Urine C5a	Kidney transplant [91]	↑ Donor urine C5a associated with recipient's delayed graft function
	FSGS [84, 87]	Urine C5a correlated with renal dysfunction, proteinuria, and interstitial fibrosis
	ANCA-associated vasculitis [83]	↑ Urine C5a in active disease vs. disease remission
Urine factor H	IgA nephropathy [92]	↑ Urine factor H ctc
	Cisplatin nephropathy [93]	↑ Urine factor H after cisplatin, correlated with lower eGFR
	Nephritis [94]	↑ Urine factor H ctc
Urine properdin	IgA nephropathy [92]	↑ Urine properdin ctc
	Kidney transplant recipients [95]	↑ Urine properdin → ↑ risk of graft failure
Urine CD59	Type 2 DM [96]	↑ → Lower risk of stage 5 CKD and death
	Membranous glomerulonephritis [97]	↑ Urine CD59 ctc
Plasma sC5b-9	Deceased donor kidney transplant recipients [98]	↑ Perioperative plasma sC5b-9 = worse graft function 1 year later
	FSGS [84]	↑ Plasma sC5b-9 ctc
Urine sC5b-9	Membranous nephropathy [84, 99, 100]	↑ Urine sC5b-9 ctc Urine sC5b-9 levels correlated with worse outcome with potential for dynamic marker of ongoing injury Urine sC5b-9 levels may identify patients with a membranous lesion

Pediatric Nephrology (2023) 38:1411–1425

Evidence of complement activation in urine sample in human AKI

- Case-control study , **AKI after cardiac surgery**:
  - **Urine factor Ba** (alternative pathway activation) **increased correlated with AKI severity**, and preceded the rise in Scr.
  - **Urine Ba may be a predictive or functional biomarker??**

Multicenter Study > Am J Physiol Renal Physiol. 2019 Sep 1;317(3):F650-F657.
doi: 10.1152/ajprenal.00130.2019. Epub 2019 Jul 17.

Urine complement activation fragments are increased in patients with kidney injury after cardiac surgery

Jennifer Laskowski [1], Heather Thiessen Philbrook [1], Chirag R Parikh [2], Joshua M Thurman [1]

Affiliations + expand

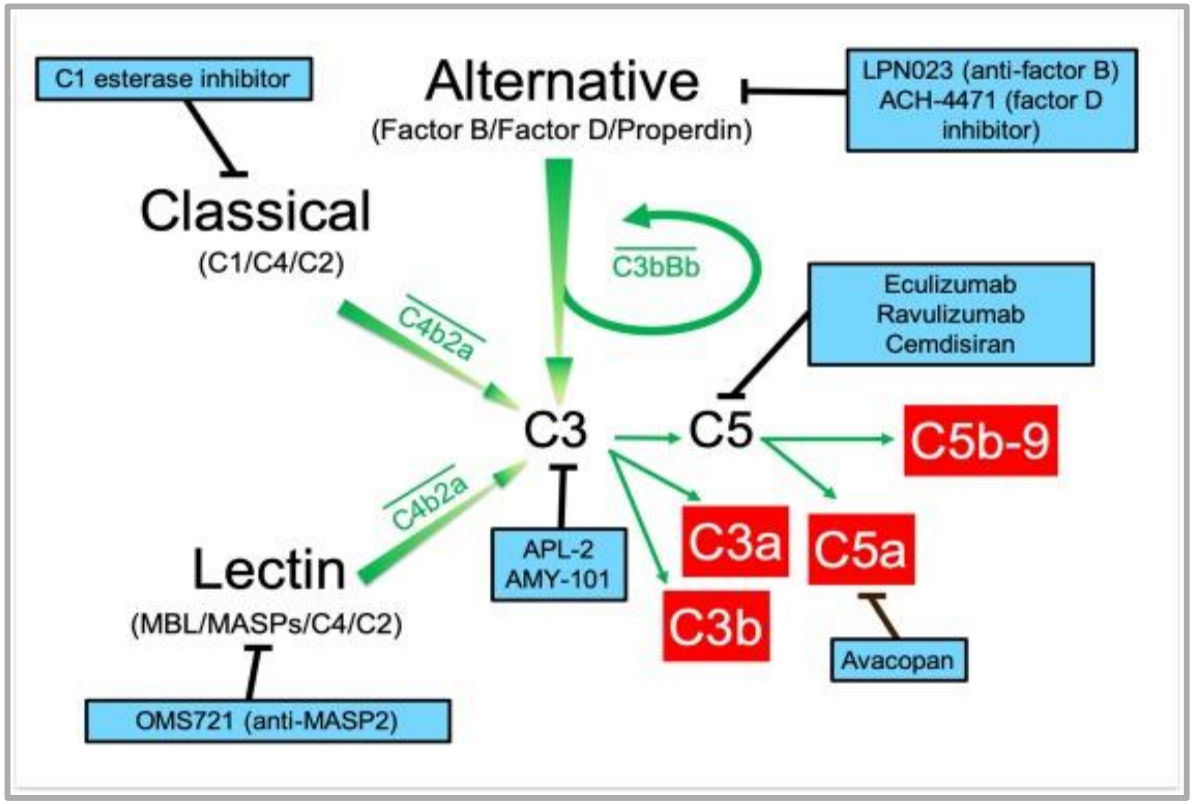
PMID: 31313951 PMCID: PMC6766629 DOI: 10.1152/ajprenal.00130.2019
Free PMC article

Abstract

Experiments in mouse models have shown that the complement cascade is activated within the kidney after ischemia-reperfusion and that complement activation contributes to tubular injury in this setting. Less is known, however, about complement activation in human kidneys after ischemia or whether complement activation in the tubulointerstitium can be detected by measurement of complement fragments in the urine. We hypothesized that urine biomarkers of complement activation would rapidly increase in patients who develop ischemic acute kidney injury, signaling complement activation within the kidney. We confirmed that the alternative pathway of complement is activated in the kidneys of mice after ischemia-reperfusion, and we found that levels of factor B fragments (generated during alternative pathway activation) rapidly increase in the urine. We next performed a case-control study in which we measured complement fragments in human urine samples from patients undergoing cardiac surgery using ELISAs. The level of Ba increased after cardiac surgery and was significantly higher in patients who developed acute kidney injury. The increase in Ba also correlated with magnitude of the subsequent rise in serum creatinine and with the need for hemodialysis during the hospitalization. These findings demonstrate that the alternative pathway of complement is activated in patients who develop acute kidney injury after cardiac surgery and that increases in the level of urine Ba may be a predictive and functional biomarker of severe kidney injury.

Keywords: acute kidney injury; complement; ischemia; kidney.

Theraputic agents blocking complement system



Poppelaars F. Molecular Immunology 2020

- **Eculizumab and ravilizumab**, monoclonal antibodies to C5, aprroved for treatment of aHUS
  - safe end effective in conjunction with meningococcal vaccination and/or prophylactic antibiotics).
- **Avacopan**, C5ar antagonist, approved for treatment of vasculitis.
- Factor B inhibitor, Factor D inhibitor and C3 inhibitor are being tested on aHUS, C3G, PNH.

Complement targeted agents for prevention of rejection in kidney transplantation

Table 1 | Complement-targeted agents for the prevention of rejection in transplantation

Compound	Entity	Target	Mechanism	Status	Refs
Intravenous immunoglobulin	Plasma protein	Multiple	Inhibits activation of complement, blocks FcR and C1q	Included in SOC	84,115–117
Rituximab	Humanized mAb	CD20 expressed on B cells	Blocks complement-induced enhancement of antibody generation	Included in SOC	115,117
C1INH	Purified or recombinant protein	C1r, C1s, MASP1, MASP2 and factor B	Inactivates complement serine proteases FXIIa, FXIa and kallikrein; blocks the classical and lectin pathways as a serpin but may also inhibit the alternative pathway by another mechanism	Clinical trials (NCT02502903, NCT01134510, NCT01147302)	90,115,117
Eculizumab	Humanized mAb	C5a	Inhibits C5 cleavage to form C5a and C5b; blocks the terminal pathway	Clinical trials (NCT01567085, NCT00670774, NCT01399593)	85,104,110,111
BIVV009	Humanized mAb	C1s	Inactivates C1s; blocks the classical pathway	Clinical trial (NCT02502903)	119,120
IdeS	Protease	IgG	Digests IgG resulting in loss of C1q binding; FcR binding is retained	Clinical trials (NCT02224820, NCT02426684, NCT02475551)	126–129
APT070 (Mirococept)	Recombinant protein (membrane-targeting truncated CR1)	C3 and C5 convertases	Inhibits C3 and C5 convertases; blocks activation downstream of C3	Clinical trial (ISRCTN49958194)	175–177
Compstatin family inhibitors	Peptide	C3	Binds to C3 and inhibits its cleavage by C3 convertases; blocks downstream activation	Clinical trial (NCT03316521)	121–123
sCR1	Recombinant protein	C3 and C5 convertases	Inhibits C3 and C5 convertases; blocks downstream activation	Preclinical development	13
TT30	Recombinant protein (chimeric CR2–factor H)	Alternative pathway C3 and C5 convertases	Binds to C3d on target cells and inhibits C3 convertases	Preclinical development	92,94
C5aR1 antagonist	Peptide	C5aR1	Blocks C5aR1 so inhibits signalling	Preclinical development	91
Cobra venom factor	Recombinant protein	C3 and C5	Forms stable alternative pathway convertase with factor B that cleaves and depletes C3 and C5	Preclinical development	148

CR2, complement receptor type 2; C1INH, C1 inhibitor; C5aR1, C5a anaphylatoxin chemotactic receptor 1; FXIa, factor XIa; FXIIa, factor XIIa; mAb, monoclonal antibody; MASP1, mannan-binding lectin serine protease 1; sCR1, soluble complement receptor type 1; SOC, standard of care.

Biglarnia AR et al, Nature Rev Nephrol 2018

- C1INH (recombinant C1 fraction inhibitor, classic and lectin pathways inhibitor) was shown to be protective against IR injury and chronic allograft fibrosis in animal and ex vivo studies.

Delpech PO Transl Med 2016

Randomized Controlled Trial › Pediatr Transplant. 2018 Mar;22(2). doi: 10.1111/petr.13129. Epub 2018 Jan 29.

A prospective randomized, controlled trial of eculizumab to prevent ischemia-reperfusion injury in pediatric kidney transplantation

Michael Kaabak [1], Nadeen Babenko [1], Ron Shapiro [1], Allan Zokoyev [1], Olga Dymova [1], Edward Kim [1]

Affiliations + expand

PMID: 29377474 DOI: 10.1111/petr.13129

Abstract

Ischemia-reperfusion injury has multiple effects on a transplanted allograft, including delayed or impaired graft function, compromised long-term survival, and an association with an increased incidence of rejection. Eculizumab, a monoclonal antibody blocking terminal complement activation, has been postulated to be an effective agent in the prevention or amelioration of IRI. We performed a single-center prospective, randomized controlled trial involving 57 pediatric kidney transplant recipients between 2012 and 2016. The immunosuppressive protocol included two doses of alemtuzumab; half of the patients were randomized to receive a single dose of eculizumab prior to transplantation. Maintenance immunosuppression was based on a combination of low-dose tacrolimus and mycophenolate, without steroids. Eculizumab-treated patients had a significantly better early graft function, less arteriolar hyalinosis and chronic glomerulopathy on a protocol biopsies taken on day 30, 1 year, and 3 years after transplantation. In the eculizumab group, four non-vaccinated children lost their grafts during the course of a flu-like infection. Eculizumab is associated with better early graft function and improved graft morphology; however, there was an unacceptably high number of early graft losses among the eculizumab-treated children. While a promising strategy, the best approach to complement inhibition remains to be established.

Keywords: alemtuzumab; eculizumab; ischemia-reperfusion injury; pediatric kidney transplantation; steroid-free immunosuppression.

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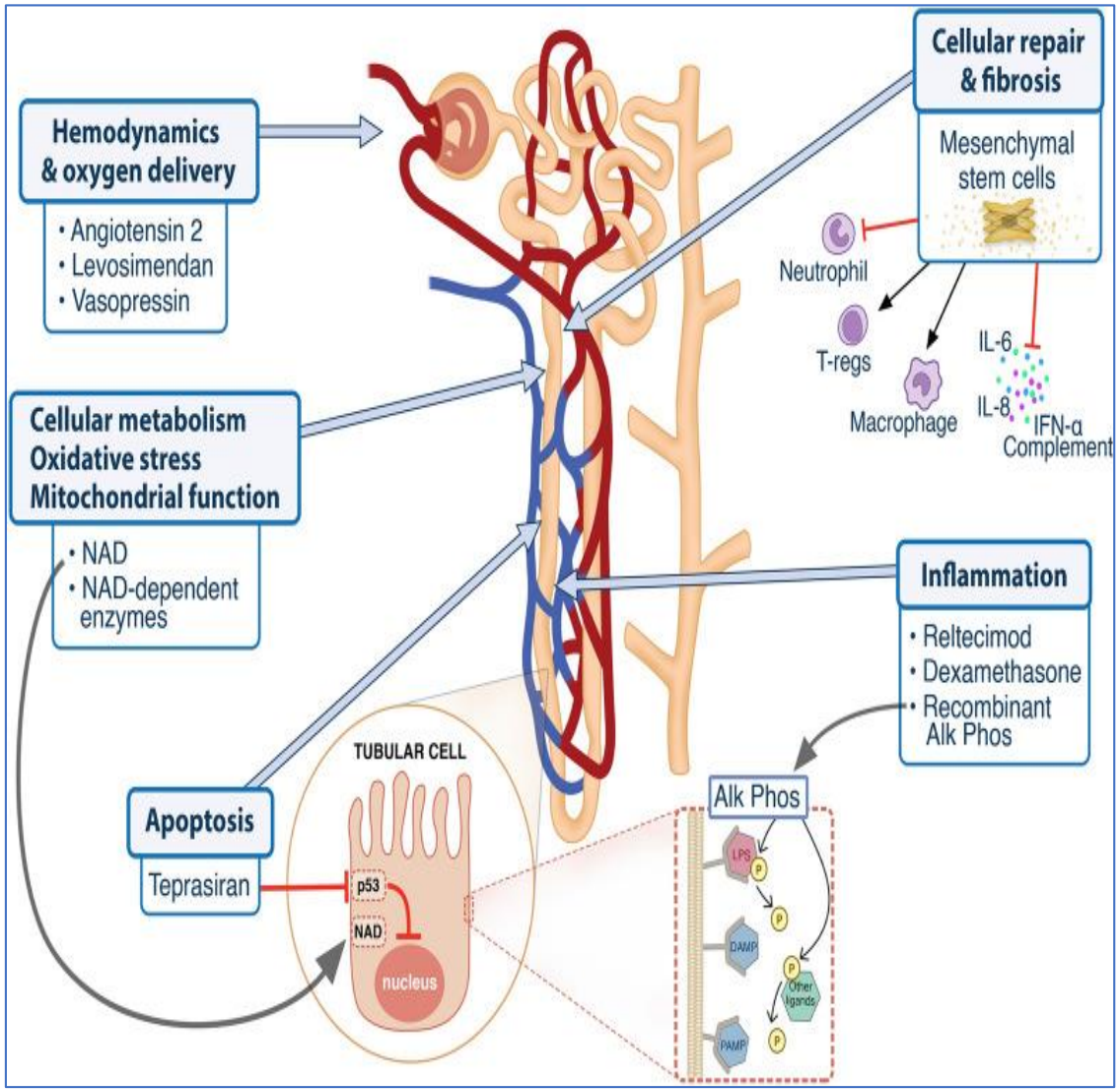
- ❑ 57 pediatric kidney transplantation recipients randomized to a **single dose of eculizumab prior to KTx**:
  - ❑ **Better early graft function**
  - ❑ **Lower arteriolar hyalinosis at subsequent biopsies**
  - ❑ **4 patients developed flu-like illness** within 60 days and **lost allografts .**

- ❑ **Peritransplant eculizumab was well tolerated** but **did not reduce the incidence of delayed graft function** (DGF) in adult deceased donor KTx recipients.

Schroppel B. Am J Transplant 2020

New drugs for acute kidney injury?

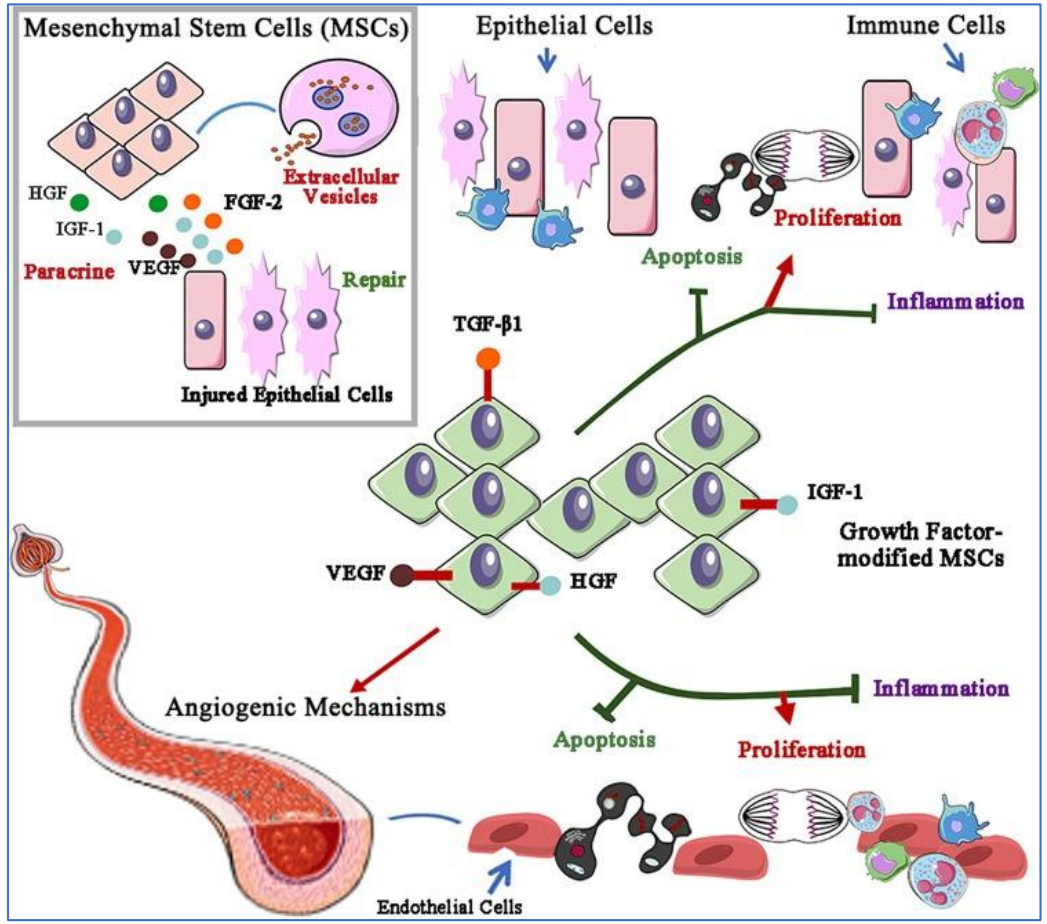


Selected compounds that impact known pathophysiological processes and have been studied in humans. *Alk Phos* alkaline phosphatase; *DAMP* danger associated molecular pattern; *IL* interleukin; *IFN-α* interferon alpha; *LPS* lipopolysaccharide; *NAD* Nicotinamide adenine dinucleotide; *T-regs* regulatory T-cells; *PAMP* pathogen associated molecular pattern

Pickkers P et al Intensive Care Med. 2022

- **Levosimendan (inodilator):** may be protective by improving cardiac function or glomerular afferent arteriolar dilatation.
  - A meta-analysis of CPB surgery patients indicated less AKI and less use of RRT. *Zhou C. AJKD 2016*
  - LEVOAKI Trial : results awaited
- **recAlkaline phosphatese**: dephosphorylates PAMPs and DAMPs.
  - Phase 2 trial sepsis-AKI: short term effects negative, longer term renal function and mortality significantly improved.
  - Phase trial REVİVAL: results awaited.
- **Sirtuins** :NAD-dependent enzymes in cellular energy metabolism and maintains undamaged DNA. Phase 2 RCT ongoing.
- **siRNA teprasiran** :Inhibits protein P53, tumor suppressor protein involved in apoptosis. Post-cardiac surgey and post-Tx: negative
- **Repair agents**: MSC therapy, Bone-morphogenic protein (BMP-7), Hepatocyte growth factor (HGF):
  - Anti-inflammatory, anti-apoptotic, anti-fibrotic, immunomodulatory, pro-angiogenic regenerative effects .
  - Favorable results with HGF-mimetic, ongoing trial in cardiac surgery and kidney transplantation
  - Inconsistent results with BMP-7 and MSC therapy

Targeted Therapies in AKI



Growth factors and stem cell-based AKI therapy. Extracellular vesicle (EV)-delivered and paracrine factors such as HGF, IGF-1, VEGF, and FGF-2 from mesenchymal stem cells contribute to repair after renal injury. More importantly, stem cells modified by growth factors, including VEGF, TGF-β1, and IGF-1, efficiently protect against AKI by decreasing apoptosis and the inflammatory response and promoting tubular epithelial and endothelial cell proliferation. VEGF-modified stem cells change capillary density via angiogenic mechanisms to attenuate renal ischemia-reperfusion injury.

- **MSC can migrate to areas of injury** and modulate local injury response by **paracrine and and ESV delivered release of soluble factos (HGF, IGF, VEGF, FGF etc).**
  - *MSC modified by HGF was shown to attenuate IR injury.*

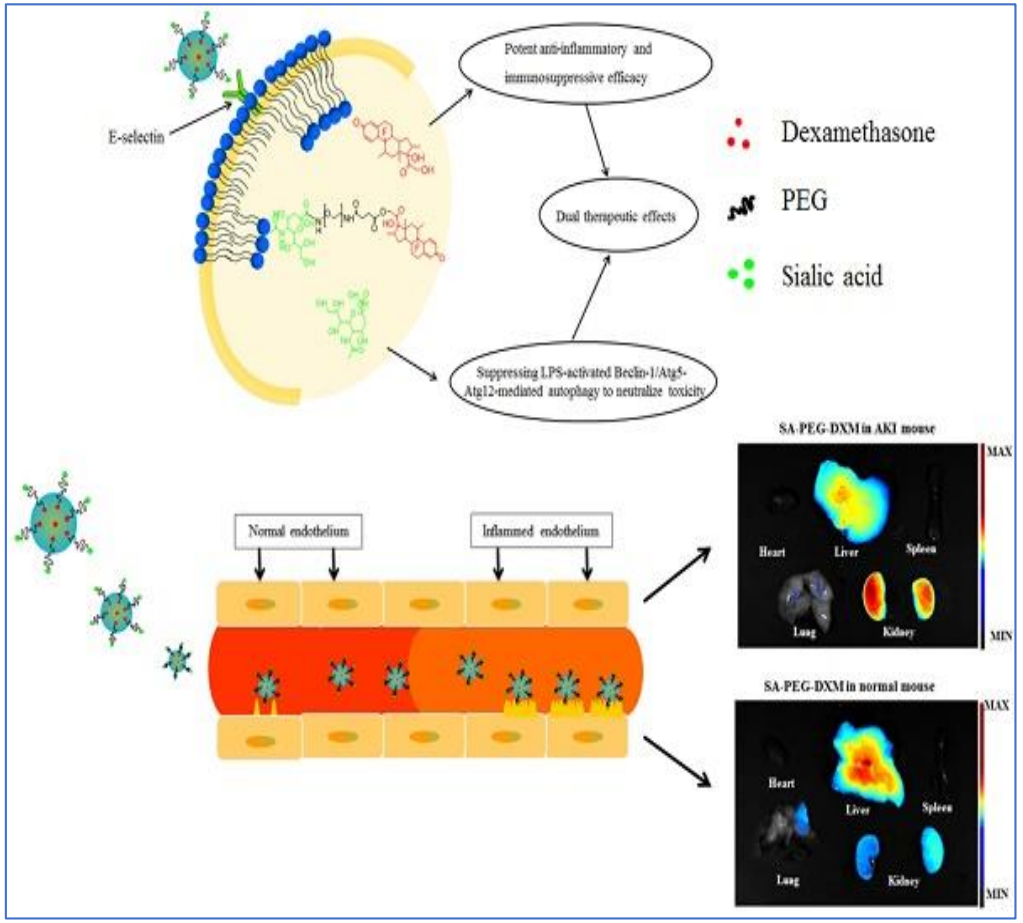
- **MicroRNA&Ektracellular Vesicles** produced and releasd by cells:
  - Membrane-bound vesicles **(exosomes and microvesicles)**
  - **contain mRNA, miRNA,** surface receptors and protein that can program the activites of other cells (**gene expression**).
  - *Endothelial-progenitor derived miR-1792 protective in IRI*

Chiba T. JASN 2021

Signal Transduct Target Ther. 2020

Nanotechnology for specific drug delivery in AKI

- **Exosome is a promising therapeutic vector to target specific drug delivery to the site of injury**.
  - Macrophage-derived micro-vesicles packed with dexamethasone effective against renal inflammation and fibrosis. *Tang T. et al. Theranostics 2019*
  - Exosomes loaded with IL-10 showed protective effects against renal IR Injury. *Tang T. Science Advances 2020*



Use of carrier systems in the renal drug delivery and strategies for the diverse target sites

Hu, JB Theranostics 2017

Conclusion

- AKI is an inflammatory syndrome with short and long-term serious complications including CKD, distant organ disease and mortality.
- Due to its multifactorial and complex nature and inability to spesifically identify AKI-phenotypes, there is lack of approved therapies in humans.
- Advances in understanding AKI pathogenesis, biomarker development and a spectrum of targeted therapies may improve prognosis in AKI.

- Thank you for your attention…