Macrophage dynamics in kidney repair:
Elucidation of a COX2 dependent MafB pathway to affect macrophage differentiation

David P. Basile[1]

[1] Department of Anatomy, Cell Biology & Physiology, Indiana University of Medicine, Indianapolis, Indiana

Address Correspondence to:

D. P. Basile

Dept. of Anatomy, Cell Biology & Physiology

Indiana Univ. School of Medicine,

635 Barnhill Dr. MS 2063

Indianapolis, IN 46202

e-mail: dpbasile@iupui.edu

Fax: 317-274-3318

This is the author's manuscript of the article published in final edited form as:

Basile, D. P. (2022). Macrophage dynamics in kidney repair: Elucidation of a COX-2–dependent MafB pathway to affect macrophage differentiation. Kidney International, 101(1), 15–18. https://doi.org/10.1016/j.kint.2021.10.020

ABSTRACT

Cylocloxygenase-2 (COX-2) is an important mediator of arachidonic acid metabolism. Pan et al., recently identified a robust increase in the expression of COX-2 in pro-resolving macrophages (M2) during the repair phase of acute kidney injury (AKI). The investigators determined the PGE2 was produced in macrophages and demonstrated that signaling through the EP4 receptor stimulated the expression of the anti-inflammatory transcription factor MafB. MafB was further shown to be essential for macrophage differentiation and mediation of the intrinsic repair response following experimental AKI.

Arachidonic acid (AA) metabolism is a complex series of processes resulting in a plethora of downstream products which influence cellular signaling and organ function. One of the primary AA pathways depends on the activity of cyclooxygenase enzymes, COX-1 or COX-2; the former is a constitutively active form, while the latter is an inducible form of the enzyme. COX activity represents the rate-limiting step in the conversion of AA to prostaglandin G2 or H2 (PGG2 or PGH2). Subsequent processing by a host of additional enzymes yields additional products such as PGE2, PGD2, PGI2 or TXA2 [1].

These COX-derived products have been the subject of extensive investigation on kidney function for many years [1, 2]. Of central interest is their purported role in regulating hemodynamics. COX-2 activity is associated with vasodilation and impairment of COX2 may result in increased vascular tone [1, 2]. In the setting of acute kidney injury (AKI), COX2 inhibition contributes to reduced medullary perfusion and an increase in susceptibility to tissue injury. Much of the COX2 mediated activity is the result of PGI2 (prostacyclin) production, and the prostacyclin analog, Iloprost, has been shown to have protective effects in models of AKI [3]. Recent studies have shown that smooth muscle specific deletion of the PGE2 receptor, EP4, results in enhanced Ang II-induced vasoconstriction and renal damage in mice [4]. These observations highlight the physiological underpinnings whereby COX2 inhibition by non-steroidal anti-inflammatory agents may predispose the development of kidney injury.

AKI is reversible, in part, to the re-establishment of renal perfusion and the intricate repair of the damaged renal parenchyma [3]. Over the past 15 years, there has been a substantial advance in our understanding of the innate immune system in both renal injury and repair. In its simplistic viewpoint, pro-inflammatory macrophages (often termed “M1”) express iNOS and secrete cytokines that promote inflammation such as TNFα, IL-1β, IL-12, IL-18 and IL-2, as well as chemokines CXC1, CXCL2, and CCL2, which are thought to recruit neutrophils [5]. Mouse models of ischemia/reperfusion (I/R) result in infiltration of these cells in the first 24 hours, where they contribute to tissue injury but their levels decline within 3 days as renal function recovers. Within this frame, the macrophage environment shifts to one dominated a pro-repair /resolving phenotype referred to as “M2”. While multiple different types of M2 macrophages have been described, these cells typically express arginase and the mannose-receptor, CD206 [5]. The overall activity of M2 macrophages reduces inflammation by inhibiting inflammatory glycoproteins, increasing production of anti-inflammatory cytokines, and the production of cell growth factors which may affect tubular proliferation [5] . With regard to effects on renal injury, the importance of these cells was documented in a classic study in which depletion of macrophages between day 3-5 post-I/R resulted in the lack of recovery from AKI [6].

However, the mechanisms mediating conversion of pro-inflammatory (M1) to the pro-resolving (M2) phenotype following kidney injury are not clear. While a variety of factors such as macrophage colony stimulating factor (M-CSF), IL-4, IL-10, IL-13, and TGF-β can stimulate M2 activation [7] the potential role that COX activity may play in the macrophage response to tissue injury or repair has not received significant attention. In this issue of *Kidney International*, Pan et al., have examined how the COX pathway in myeloid cells alters the balance between tissue repair and damage following acute injury [8] . The authors demonstrated that macrophage COX-2 expression is unaltered in the first day following renal I/R but rises dramatically by *day-3*. This switch to increased COX-2 expression parallels the known shift from a predominantly M1 to M2 environment and prompted these investigators to hypothesize that COX2 mediates differentiation into a pro-resolving phenotype. This hypothesis is consistent with other reports suggesting that COX2 may modulate the M1 to M2 transition in other models [9].

In the current study, myeloid specific COX-2 knockout mice (CD11bCre; Cox2 $^{fl/fl}$) were used to evaluate the role of this pathway following renal I/R. While wild-type (WT) mice show reduced renal function within 1 day of I/R, resolution of injury is apparent within 3 days. However, myeloid-specific COX-2 KO mice failed to fully recover renal function and had sustained neutrophil infiltration relative to WT mice. The macrophages from COX-2 KO expressed higher levels of pro-inflammatory genes relative to WT mice, and were less efficient in the ability to clear neutrophils by efferoptosis. Thus, myeloid COX-2 activation may potentiate differentiation of macrophages to a pro-repair, M2-like phenotype. It has been shown that the lack of an efficient repair predisposes the development of CKD and renal fibrosis [3]. It is therefore noteworthy that macrophage-specific COX2 KO mice also develop significant CKD at time points up to 4 weeks following I/R and enhanced renal fibrosis following unilateral ureteral obstruction (UUO).

The investigators then took taking advantage of COX2 KO derived macrophages and identified that PGE2 represents the major prostanoid product generated from AA. PGE2 binds and activates several G-protein coupled receptors referred to as prostaglandin E2 receptors, EP1-4, which are encoded by *Ptger1, Ptger2, Ptger3* and *Ptger4* genes. The investigators proposed that EP4 receptors may be important in macrophage differentiation, since only *Ptger4* mRNA was responsive to macrophage polarization stimuli *in vitro.* Moreover, the EP4 antagonist, L161982 blocked macrophage polarization *in vitro*, while EP2 antagonists did not.

EP4 is a G-protein coupled receptor leading to the activation of the cAMP/PKA/CREB signaling pathway and this activity is thought to participate in macrophage differentiation. However, *in silico* analysis could not identify CREB binding sites on the promoters of PGE2-induced anti-inflammatory genes. MafB is an anti-inflammatory transcription factor that has been suggested to mediate M2 polarization [10]. Thus, the authors sought to evaluate whether MafB may mediate PGE2 activity in macrophages. Using Gene Set Enrichment Analysis (GSEA) on data sets from PGE2 stimulated macrophages in combination with data sets to identify MafB target genes, the authors found overlap in the transcriptional response of PGE2 and MafB. They suggest MafB can stimulate expression of *Arg1*,*Tgm2*, CD206 and Il10 and repress the activation of pro-inflammatory genes such as *Tnf, Ccl2 and Ccl3*. Evidence that PGE2 can activate MafB activity was confirmed using a MafB promoter/reporter transfected into RAW264.7 cells. The promoter activity of this construct was activated by PGE2, as well as the EP4 agonist CY10598, and blocked by an EP4 antagonist.

Taken together, the data support the existence of a pathway modulating macrophage differentiation to a pro-resolving phenotype based on the activity of COX2> PGE2> EP4> MafB>anti-inflammatory gene activation **(Figure 1)**. Consistent with this proposal, MafB was localized in macrophages of WT mice at 3 days following renal injury but not observed in myeloid-specific COX2 KO mice. This was further supported by studies in which myeloid specific knockout of either EP4 or MafB resulted in a similar lack of recovery following I/R injury as myeloid COX2 KO mice. Interestingly, the investigators demonstrated that an EP4 agonist (ONO-4819) could increase MafB expression in macrophages of COX2-/- mice and “rescue” the phenotype by improving recovery following renal I/R.

The study is noteworthy insofar as the investigators utilized an impressive combination of biochemical, transgenic and *in silico* experimental approaches to convincingly map a specific pathway involved in the endogenous repair of the kidney. It adds physiological context into the mechanisms of macrophage regulation, which is not well understood despite being subject of intensive research efforts in the kidney repair literature. The important results of this study, open new major questions regarding this pathway that should be addressed. For example, what is the basis for the increase in macrophage COX2 expression during the recovery phase

post-ischemia, and how is this pathway is modified by complicated milieu of the injured kidney? Importantly, COX2 expression was ~100-fold higher in macrophages relative to the total kidney tissue. This observation is important since the ability to target and activate a specific cell type would represent an ideal strategy to hasten resolution of injury.

The study also provides food for thought regarding the complicated role of COX2 in renal pathophysiology. Given that this pathway is often targeted for treatment of pain and inflammation, these studies highlight another possible mechanism by which COX-2 inhibitors may be contra-indicated in conditions associated with development or recovery from AKI.

Conversely, the study highlights that specific PGE2-mediated pathways may provide benefit in improving the efficacy of repair. The current study demonstrated EP4 agonists reversed the repair defect in mice with COX2 deficient macrophages. However, whether EP4 agonists would improve the repair in WT mice was not addressed in the current study. Theoretically, EP4 activation represents a strategy to amplify a beneficial pro-repair pathway without altering other elements of the complicated AA system. Several EP4 agonists have been developed and used to resolve inflammation in a variety of experimental settings including pre-clinical models of osteoarthritis [11], and ulcerative colitis[12]. Another recent study has shown that EP4 agonists can attenuate hypertension in the salt-sensitive Dahl-S rat model[13]. The current study has the potential to stimulate further translational research to investigate this pathway as a potential therapeutic target following kidney injury.

REFERENCES

1. Imig JD. Eicosanoids and renal vascular function in diseases. *Clin Sci* 2006; **111:** 21-34.

2. Harris RC, Breyer MD. Update on Cyclooxygenase-2 Inhibitors. *Clinical Journal of the American Society of Nephrology* 2006; **1:** 236-245.

3. Basile DP, Anderson M, Sutton TA. Pathophysiology of acute kidney injury. *Comprehensive Physiology* 2012; **2:** 1303-1353.

4. Thibodeau JF, Holterman CE, He Y*, et al.* Vascular Smooth Muscle-Specific EP4 Receptor Deletion in Mice Exacerbates Angiotensin II-Induced Renal Injury. *Antioxidants & redox signaling* 2016; **25:** 642-656.

5. Han HI, Skvarca LB, Espiritu EB*, et al.* The role of macrophages during acute kidney injury: destruction and repair. *Pediatr Nephrol* 2019; **34:** 561-569.

6. Lee S, Huen S, Nishio H*, et al.* Distinct Macrophage Phenotypes Contribute to Kidney Injury and Repair. *Journal of the American Society of Nephrology* 2010; **22:** 317-326.

7. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nature reviews Immunology* 2008; **8:** 958-969.

8. Pan Y, Cao S, Terker A*, et al.* Myeloid COX-2/PGE/EP4 promotes MafB-dependent inflammatory resolution in acute kindey injury: PGE modulates macrophage phenotype through MafB. *Kidney International* 2021; **In press**.

9. Ho V, Rauh M, Krystal G. COX-2 inhibition represses the generation of M2 macrophages and slows tumor progression (111.31). *The Journal of Immunology* 2011; **186:** 111.131-111.131.

10. Kim H. The transcription factor MafB promotes anti-inflammatory M2 polarization and cholesterol efflux in macrophages. *Scientific Reports* 2017; **7:** 7591.

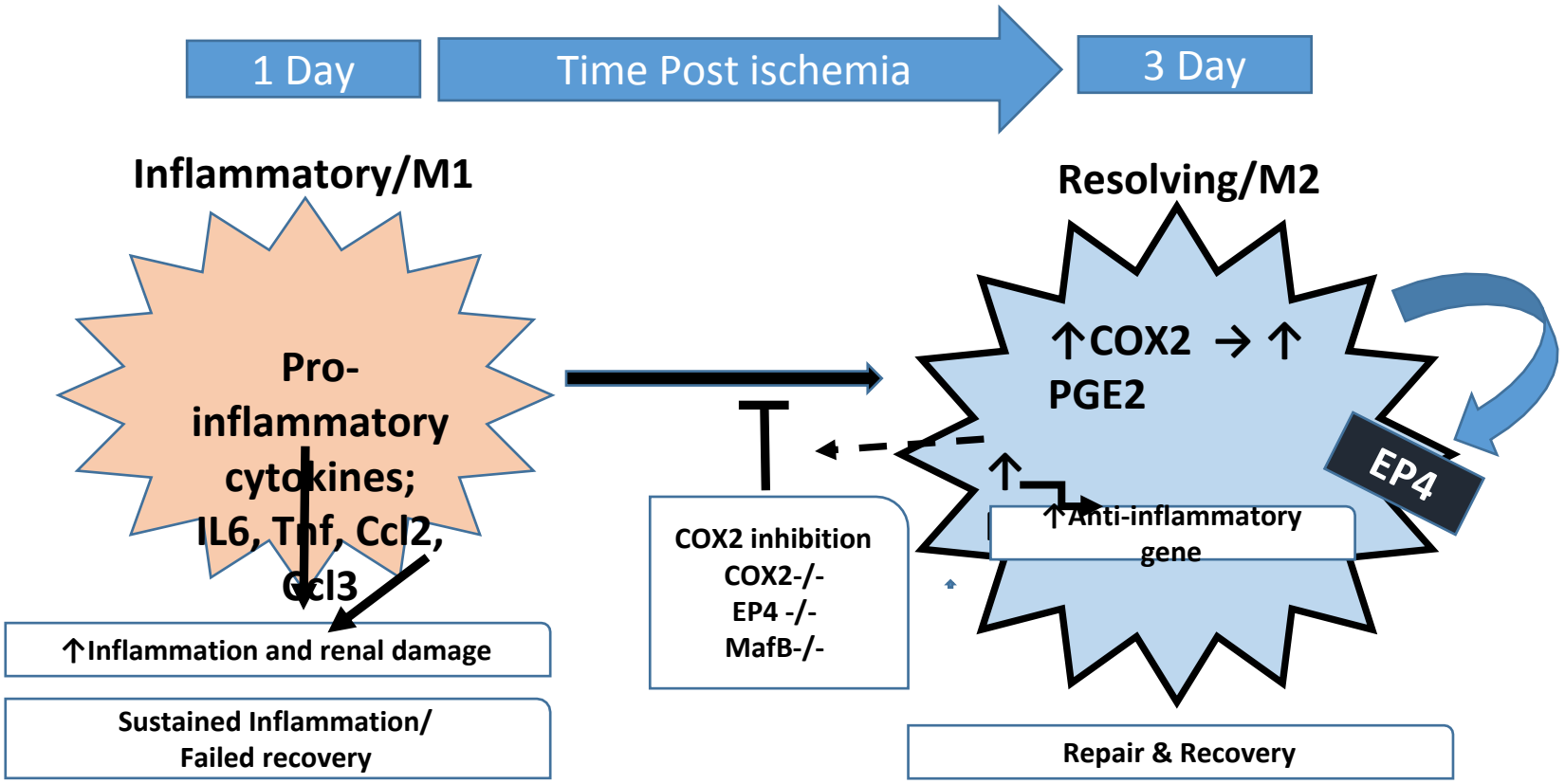
11. Murahashi Y, Yano F, Chijimatsu R*, et al.* Oral administration of EP4-selective agonist KAG-308 suppresses mouse knee osteoarthritis development through reduction of chondrocyte hypertrophy and TNF secretion. *Scientific Reports* 2019; **9:** 20329.

12. Watanabe Y, Murata T, Amakawa M*, et al.* KAG-308, a newly-identified EP4-selective agonist shows efficacy for treating ulcerative colitis and can bring about lower risk of colorectal carcinogenesis by oral administration. *European journal of pharmacology* 2015; **754:** 179-189.

13. Green LA, Njoku V, Mund J*, et al.* Endogenous Transmembrane TNF-Alpha Protects Against Premature Senescence in Endothelial Colony Forming Cells. *Circulation research* 2016; **118:** 1512-1524.

FIGURE LEGEND

Figure 1. Schema outlining COX2 effects of macrophage differentiation facilitating renal repair. Activation of M1 macrophages in the early stages of ischemic injury results in pro-inflammatory tissue injury. The proposed pathway is based on the development of myeloid COX2 activity which generates PGE2. This prostinoid activates the EP4 receptor resulting in in the activation of the MafB transcription factor and subsequent activation of a pro-resolving phenotype associated with tissue repair. Attenuation of the pathway, results in sustained M1 activity, failure to resolve inflammation and sustained renal damage leading to chronic kidney disease.


1 Day
Time Post ischemia
3 Day
Inflammatory/M1
Resolving/M2
Pro-inflammatory cytokines; IL6, Tnf, Ccl2, Ccl3
↑COX2 → ↑ PGE2
EP4
↑Anti-inflammatory gene
COX2 inhibition
COX2-/-
EP4 -/-
MafB-/-
↑Inflammation and renal damage
Sustained Inflammation/ Failed recovery
Repair & Recovery