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CSF-1 signaling suppresses renal crystal formation

Kazumi Taguchi[*], Atsushi Okada[*], Hiroshi Kitamura[†], Takahiro Yasui[*], Taku Naiki[*], Shuzo Hamamoto[*], Ryosuke Ando[*], Kentaro Mizuno[*], Noriyasu Kawai[*], Keiichi Tozawa[*], Kenichi Asano[‡], Masato Tanaka[‡], Ichiro Miyoshi[†], Kenjiro Kohri[*]

**Department of Nephro-urology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.*

†Department of Comparative and Experimental Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

[‡]Department of Laboratory of Immune Regulation School of Life Science, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan.

ABSTRACT

We previously used microarray analysis to demonstrate a correlation between renal calcium oxalate (CaO_x) crystal formation and macrophages (Mφs). We also observed the migration of Mφs to renal tubular cells (RTCs) that include crystals, followed by the disappearance of crystals in the tubular lumen from the corticomedullary junction in hyperoxaluric mice. Mφs and multinucleated giant cells also encapsulated interstitial crystals in the kidneys of hyperoxaluric rats and in humans with acute and chronic oxalosis. In addition, normal rat kidney epithelial cells expressed monocyte chemotactic protein-1 (MCP-1), the levels of which increased following exposure to CaO_x monohydrate (COM) crystals and oxalate. Finally, several *in vitro* and *in vivo* studies reported that urinary COM crystals could be degraded and dissolved by Mφs. These findings suggest that Mφs influence crystal processing, although the mechanism(s) by which they interact with crystals remain unclear.

There are 2 subtypes of Mφs, classically activated (M1) and alternatively activated (M2) Mφs, which exert pro- and anti-inflammatory effects, respectively. Resident tissue Mφs differentiate after stimulation by various cytokines. Associations were reported between kidney stones and atherosclerotic plaques in which various types of Mφ migrate. In these studies, M2Mφs infiltrated early

atherosclerotic lesions, whereas M1Mφs were found predominantly in advanced lesions. Plaque formation correlated with the dominance of the M1 phenotype. Several reports suggested that M2Mφs decreased tissue inflammation and promoted tissue healing in glomerulonephritis and in ischemia/reperfusion kidneys.

Some reports suggested that CSF-1 signaling mediates tissue regeneration after injury, and also alters Mφ polarization towards the M2 phenotype. In addition, several studies have used CSF-1-deficient (*op/op*) mice, which contain a point mutation in *CSF1* and exhibit fewer circulating monocytes and tissue Mφs than mice with CSF-1. Although these studies demonstrated that loss of CSF-1 significantly reduces atherosclerotic severity, they did not elucidate the role of Mφ polarization in these outcomes.

In this study, we examined and compared renal crystal formation and Mφ polarization in wild-type and CSF-1-deficient mice after hyperoxaluric treatment. The amount of renal calcium oxalate crystal deposition in CSF-1-deficient mice was significantly higher than in non-deficient mice. CSF-1 treatment increased the expression of M2-related genes and markedly decreased the number of renal crystals in both CSF-1-deficient and wild-type mice. Flow cytometry of

sorted renal Mφs showed that CSF-1 deficiency resulted in a smaller population of $CD11b^{+}F4/80^{+}CD163^{+}CD206^{hi}$ cells, which represent M2-like Mφs. In addition, M2Mφ transfusion to CSF-1-deficient mice suppressed renal crystal deposition directly. An *in vitro* crystal phagocytosis assay demonstrated that the capacity of calcium oxalate monohydrate (COM) crystals to undergo phagocytosis was higher in M2- than M1-polarized Mφs and renal tubular cells. Gene array profiling showed that CSF-1-deficiency resulted in disordered M2- and stone-related genes. Collectively, our results provide compelling evidence for a suppressive role of CSF-1 signaling in renal crystal formation.