MINIREVIEW

An inflammatory nexus: Serum amyloid A and inflammation in diabetic kidney disease

Robert J. Anderberg[1], Brad P. Dieter[1], Rick L. Meek[1], Katherine R. Tuttle[1,2]

[1]*Providence Medical Research Center Providence Health Care Spokane, Washington, USA*
[2]*University of Washington School of Medicine Seattle, Washington, USA*

Correspondence: Robert J. Anderberg
E-mail: bob.anderberg@providence.org

Inflammation contributes a significant part to the advancement of diabetic kidney disease (DKD), yet relatively little is known about the root cause of these inflammatory events. Serum Amyloid A (SAA) triggers a potent inflammatory response in a variety of tissues and is up-regulated in glomerular and tubulointerstitial compartments of the diabetic kidney. Under inflammatory conditions, podocytes, along with other intrinsic cells, produce SAA locally in the kidney. Our recent work has shown that SAA induces NF-κB activation and subsequent inflammatory chemokines and cytokines in cultured podocytes. Recent evidence suggests that local production of SAA in diabetes may lead to monocyte and macrophage recruitment, neutrophil activation, and other related incidents resulting in sustained chronic inflammatory conditions in the kidney which may further exacerbate DKD.

Keywords: Leukocytes; monocytes; macrophages; chemokines; cytokines; advanced glycation end products; nuclear factor kappa-B

Abbreviations: DKD, diabetic kidney disease; SAA, Serum Amyloid A; AGE, advanced glycation end products; RAGE, advanced glycation end product receptor; MCP-1, monocyte chemoattractant protein-1; MIP-3, Macrophage inflammatory protein-3-alpha; ENA-78, epithelial-derived-neutrophil-activating protein; C3, Complement component 3

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SAA in diabetic kidney disease

Diabetic kidney disease (DKD) is recognized as an inflammatory disease due to robust evidence that leukocytes are recruited to and cause injury in kidney tissue of patients with DKD [1, 2]. Acute-phase serum amyloid A (SAA) has been implicated in promoting inflammation in a variety of tissues [3-5]. We have recently demonstrated that SAA is elevated in the plasma and kidneys of both people with DKD and analogous mouse models [3]. A mechanism for this could be production of SAA by exposure of the kidney, and other tissues and organs, to advanced glycation end products (AGE), anomalous metabolic by-products that are elevated in the diabetic state and promote oxidative stress and inflammation [6-8]. Our research has shown that AGE induce SAA up-regulation in glomerular podocytes through the advanced glycation end product receptor (RAGE) [8]. This receptor is responsible for initiation of a variety of inflammatory events in DKD, including the attraction of leukocytes to the glomerulus [9]. RAGE is known to activate NF-κB, a central transcription factor that elevates expression of a variety of cytokines and chemokines and is up-regulated by SAA *in vitro* [3, 10]. NF-κB has been directly implicated in contributing to DKD through the promotion of macrophage infiltration in kidneys of diabetic mouse models [11]. Indeed, human patients with DKD showed a direct correlation of activated NF-κB levels in peripheral blood mononucleocytes compared to control subjects. In addition, NF-κB-controlled promoters are up-regulated in patients with DKD [12, 13]. Podocytes exposed to SAA are potential intermediaries for NF-κB-mediated inflammation in DKD, which parallels other recent findings showing that podocytes produce
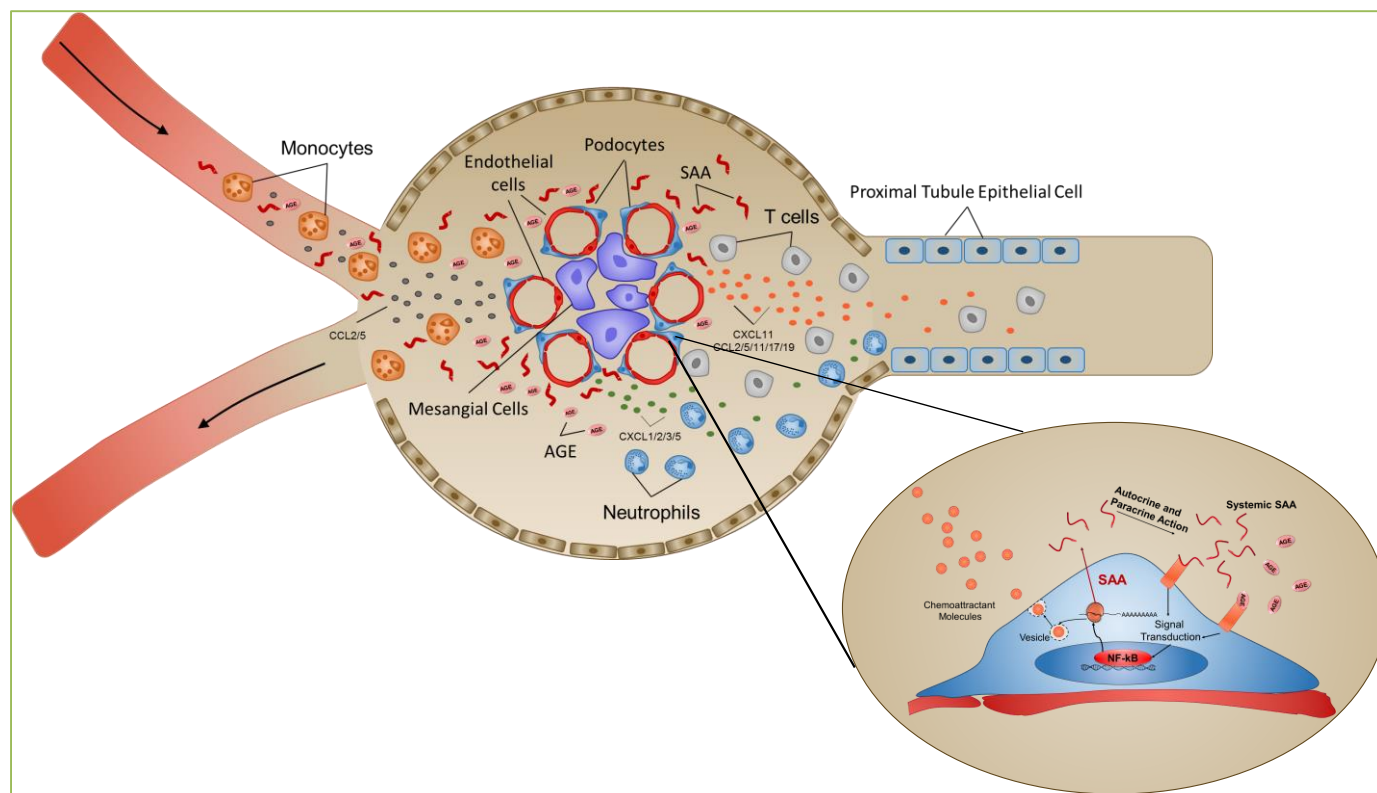
Figure 1. Model of SAA-induced inflammation response. Exposure to diabetes-like conditions causes increased production of SAA by key kidney cells, including podocytes, mesangial cells, and tubular epithelial cells. In turn, SAA induces expression of a host of chemokines that recruit leukocytes, including monocytes, neutrophils, T-cells, and dendritic cells to the glomerulus.

inflammatory mediators that contribute to self-injury by autocrine mechanisms [3, 14, 15].

Exposure of podocytes to SAA induces a major inflammatory response triggering NF-κB-mediated transcription of a wide variety of cytokines and chemokines, including SAA itself [3]. A repercussion of AGE/RAGE-mediated up-regulation of SAA is that SAA could be a source of persistent diabetes-induced chronic inflammation in the kidney through a forward-feeding autocrine loop (Figure 1) [16]. We hypothesize that such a feed-forward loop may be a manifestation of epigenetic-independent "metabolic memory," arising from aberrant glycemic metabolism resulting in perpetual inflammation via SAA activation. Thus, even with restoration of normoglycemia and dietary interventions to reduce pathological metabolites such as AGE, the inflammatory cycle may persist unabated.

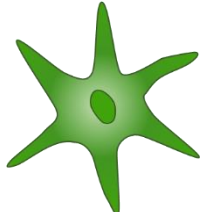## SAA-induced expression of pro-inflammatory factors

Our research demonstrated that several classes of proteins were up-regulated by exposure to SAA in cultured podocytes [3]. Several of these have been directly implicated in DKD-related inflammation. One class is the CC chemokine family. Perhaps the most well-known is monocyte chemoattractant protein-1 (MCP-1, or Ccl2). One of the functions of MCP-1 is to stop rolling monocytes by arresting them to vascular endothelium under blood flow conditions, indicating that increased production of MCP-1 in cells promotes accumulation of monocytes in tissues [17]. MCP-1 has been shown to correlate with macrophage accumulation and kidney injury in a diabetic mouse model [18]. Subsequent studies have shown that RAGE-induced MCP-1 contributes to initiation and progression of kidney damage in diabetic mice and also correlates with advanced tubulointerstitial lesions in human DKD [19-22].

Several other CC chemokines are also up-regulated in podocytes exposed to SAA [3]. Like MCP-1, the chemokine RANTES or Ccl5 stops rolling monocytes attached to vascular endothelium under flow conditions [23]. RANTES is known to be expressed in the kidney of nephritic mouse models, recruits T-cells and initiates other immune responses, causing injury to tissues [24]. Macrophage inflammatory protein-3-alpha (MIP-3 or Ccl20) is up-regulated in proximal tubular cells under high-glucose conditions and appears to be dependent on transforming growth factor-β1, known to play a key role in regulating inflammation and fibrosis under diabetic conditions [25].

Members of the CXC chemokine family are also

Table 1. SAA-induced chemokines and known target cells

Chemokines	Cell Type
Cxcl1/2/3/5/	Neutrophils
Ccl2/5	Monocytes
Cxcl 11 Ccl2/5/11/17/19	T-Cells
C17/19	Dendritic Cells

up-regulated by exposure of podocytes to SAA [3]. One of these is epithelial-derived-neutrophil-activating protein (ENA-78 or Cxcl5). Increased expression of ENA-78 in the tubular epithelium has recently been shown to promote neutrophil accumulation and contribute to kidney damage in kidneys of mice with glomerulonephritis [26]. Furthermore, human subjects with type 2 diabetes and DKD have increased urinary levels of ENA-78 compared to non-diabetic patients with other kidney diseases, suggesting that ENA-78 specifically contributes to inflammation in DKD [27]. Other members of the CxC family that are also up-regulated in podocytes by SAA include: Cxcl1, Cxcl2, Cxcl3 and Cxcl11 [3]. Atherosclerotic plaques are a known source of elevated SAA expression, and the receptor CxcR2, whose ligands include Cxcl1, 2, 3, and 5, mediates macrophage accumulation in these plaques [28, 29]. Cxcl1 is transcriptionally up-regulated in kidneys of mice and humans with DKD, while Cxcl2 has been implicated in protein kinase Cβ-mediated kidney damage in diabetic mice [30-32]. Cxcl11 has also been shown to be up-regulated in the tubulointerstitium of people with DKD [32]. This ligand binds the CxcR3 receptor, which has been found to be expressed on mesangial cells in people with glomerulonephritis, and is thought to be involved in mesangial cell proliferation.[33] Blocking this receptor inhibited leukocyte recruitment under inflammatory conditions [34]. SAA also promotes chemotaxis of monocytes and dendritic cells by activating a synergistic CC and CxC chemokine axis [35]. Therefore, it is likely these chemokines work to facilitate leukocyte recruitment and mediate SAA-related inflammation in the kidney.

In addition to leukocytic chemokines, other pro-inflammatory factors associated with diabetic kidney injury are expressed in SAA-exposed podocytes [3]. Complement component 3 (C3) plays a central role in complement activation by both the classical and alternate pathways. There is clear consensus that the dysregulation of the alternate complement pathway, leading to increased deposition of C3 without immunoglobulins in the glomerulus, is involved in pathogenesis of a class of kidney diseases termed C3 glomerulopathy [36]. In addition to classical C3 production by the liver and mononuclear cells, we recently discovered that C3 is expressed by podocytes and markedly up-regulated by SAA exposure [3]. Moreover, hyperglycemia-dependent glomerular C3 deposition occurred concurrently with development and progression of glomerular injury in the *db/db* mouse model of DKD [18]. Notably, these data in experimental models are corroborated by transcriptomic data that demonstrate increased C3 expression in kidneys of humans with DKD [32].

SAA and local tissue inflammation

The production of numerous pro-inflammatory factors by kidney cells exposed to SAA may be a significant contributor to tissue inflammation. As different cytokines and chemokines attract different cell-types, the myriad factors we found to be increased in the podocyte response to SAA exposure are likely to result in accumulation of different cell types, including monocytes, neutrophils, T-cells, and dendritic cells in the glomerulus and tubulointerstitium (Table 1 and Figure 1). A recent study in SAA3 knockout mice has been particularly enlightening and provides evidence to support our hypothesis that SAA is a major contributor to local inflammation and leukocyte recruitment. SAA3 knockout (SAA3$^{-/-}$) C57BL/6 mice fed a pro-inflammatory high-fat, high-sucrose diet showed decreased expression of MCP-1 and also the inflammatory mediator tumor necrosis factor in visceral adipose tissue [37]. Additionally, macrophage accumulation in visceral adipose tissue was attenuated [37]. Although data on the kidneys of these mice were not reported, these results support the general concept that SAA can increase local tissue inflammation and macrophage recruitment.

Conclusions

It is well established that expression of chemoattractant factors and accumulation of leukocytes in kidney tissue are major contributors to DKD. Given our recent findings showing elevated SAA in the blood and kidney tissue (glomerular and tubulointerstitial compartments) of patients with DKD, along with our data demonstrating that SAA induces robust expression of a host of chemoattractant molecules in podocytes, it is likely that SAA promotes inflammation in DKD. We propose the following conceptual model: Diabetes results in production of serum amyloid A in kidney cells via an AGE-RAGE signaling pathway. Local production of SAA results in production of chemoattractant and pro-inflammatory factors including SAA itself. The eventual result is a feed-forward signaling loop of SAA that perpetuates autocrine expression by podoyctes, leading to continuing RAGE activation, leukocyte recruitment, and inflammation in the diabetic kidney (Figure 1). Future therapies targeting SAA may attenuate a key mediator in the production of inflammation, providing a promising avenue to reduce inflammation and preserve the kidney in diabetes.

Conflict of Interest

The authors declare that there is no conflict of interest.

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