Review

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Review

Type I IFN in Glomerular Disease: Scarring beyond the STING

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Abstract: The field of nephrology has recently directed a considerable amount of attention towards the stimulator of interferon genes (STING) molecule since it appears to be a potent driver of chronic kidney disease (CKD). STING and its activator, the cyclic GMP-AMP synthase (cGAS) are among the most relevant inducers that promote the expression of type I interferons (IFN-Is). These cytokines have been long recognized as part of the mechanism used by the innate immune system to battle viral infections; however, their involvement in sterile inflammation remains unclear. Mounting evidence pointing to the involvement of the IFN-I pathway in sterile kidney inflammation provides potential insights into the complex interplay between the innate immune system and damage to the most sensitive segment of the nephron, the glomerulus. The STING pathway is often cited as the cause of renal disease originating from non-infection related caused by the induction of IFN-I expression. However, other receptors have been implicated in this process, mainly by recognizing host-derived nucleic acids. This review explores the main endogenous inducers of IFN-I in glomerular cells, discusses what exposure to the main autocrine and paracrine cytokines has on these cells, and identifies the pathways that are implicated in the development of glomerular damage.

Keywords: glomerulonephritis; IFN-I pathway; intracellular pattern-recognition receptors; STING; sterile inflammation

1. Introduction

Over one hundred years ago, Paul Ehrlich introduced the term "horror autotoxicus" to the field of immunopathology. He used this term to refer to the process whereby the immune system directs its attention to attack itself. This term finds increased relevance today and can be used to describe many types of hypersensitivity reactions, including autoimmune diseases (types II and III) and disorders stemming from a hyperinflammatory response, including cytokine storm syndrome and other autoinflammatory diseases.

Recently, molecules typically associated with an antiviral immune response, cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING), have been linked to the development of chronic kidney diseases (CKD) without involving infection. Instead, activation is through host-derived endogenous nucleic acids. Most relevant to potential therapeutic intervention, STING blockade results in reduced kidney damage,[1] especially at the level of the glomerulus.[2,3] Interestingly, although the expression of type I interferons (IFN-I) is one of the expected outcomes after the activation of cGAS/STING and other intracellular pattern-recognition receptors (PRRs), it is still not clear if the renoprotective effects of cGAS/STING blockade rely on the subsequent abrogation of IFN-I production more than other involved mechanisms.

Glomerulonephritis (GN) encompasses a group of immune-mediated diseases characterized by inflammation and fibrosis within the glomerulus causing well-described histopathological lesions that are used for clinical classification. Seven major categories are included in GN and include: 1)

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membranoproliferative GN (MPGN), 2) minimal change disease (MCD), 3) focal segmental glomerulosclerosis (FSGS), 4) membranous nephropathy (MN), 5) IgA nephropathy (IgAN), 6) crescentic GN (CGN), and 7) lupus nephritis (LN).[4] Although the current classification is helpful for diagnosis, several and often different etiologies are involved in the development of each. Indeed, among the different etiologies, infections are responsible for only a fraction of GN cases while most are caused by a) endogenous factors often involving auto-antibodies against a broad spectrum of glomerular cell-specific molecules, b) genetic defects leading to autoinflammatory disorders, and c) as a consequence of other non-communicable diseases such as diabetes.[5] Taken together, this group of diseases is part of the above mentioned "horror autotoxicus".

Among the different molecular mechanisms involved in the pathogenesis of GN, there is accumulating evidence that the pathways that induce IFN-I expression along with the pathways activated downstream of IFN-I recognition (hereafter both referred to as the IFN-I pathway) participate in the progression of glomerular damage.

Much evidence stems from patient data derived from kidney biopsies, identifying the upregulation of gene products involved in the IFN-I pathway. For example, patients diagnosed with C3 glomerulopathy MPGN show an up-regulation of IFI44L, IFIT1, MX1 and OAS2 genes,[6] and STING was observed to be over-expressed in MCD and IgAN.[7] Using a weighted gene co-expression network analysis (WGCNA), FSGS patients show an enrichment in the IFN-I signaling pathway in glomerular endothelial cells,[8] while anti-PLA2R MN patients show a similar enrichment in the IFN-I pathway using gene ontology (GO) analysis in mesangial cells. Specifically, these patients show increased expression of IFI6, a gene stimulated after IFN-I recognition.[9] In LN, the IFN-I pathway is consistently recognized as a key marker of the disease in kidney and other tissues, with immune cells being the main contributors of the activated IFN-I pathway.[10]

The most compelling evidence suggesting the direct involvement of the IFN-I pathway in the development and progression of GN comes from patients receiving recombinant IFN therapy or were discovered to have genetic alterations that cause enhanced IFN-I production. Of note, patients diagnosed with multiple sclerosis, hepatitis C, some types of cancer, and other diseases developed glomerular lesions after received recombinant IFN-I (rIFN-I) therapy.[11-14] Among the inborn errors causing enhanced IFN-I production are Aicardi-Goutières syndrome,[15] STING-associated vasculopathy with onset in infancy (SAVI),[16,17] and systemic lupus erythematosus (SLE).[18] These genetic diseases all present with glomerular injury.

Results from experiments using animal models have provided additional support for the direct effect of IFN-I on the glomerulus. A murine model of nephritis generated by the injection of anti-glomerular basement membrane (GBM) antibodies showed an increase in IFNα in renal tissue and correlated with renal dysfunction demonstrated by increased levels of blood urea nitrogen (BUN) and proteinuria. Moreover, the exogenous overexpression of IFNα using an adenoviral vector increased proteinuria and lead to a higher GN score in treated animals.[19] In a similar approach, Migliorini *et al*. combined a model of adriamycin nephropathy with the administration of IFNα and IFNβ and showed enhanced kidney dysfunction through the perturbation of parietal epithelial cells (PEC) and podocyte loss.[20] In testing a novel model of murine SLE, Nacionales *et al.* showed that blocking IFN-I activity by knocking out an IFN-I receptor (IFNAR), kidney dysfunction and glomerular injury could be ameliorated.[21] Taken together, results from these experiments suggest that the different causes of GN drive a diverse set of complex immunopathologies, including the stimulation of the IFN-I pathway which, in turn, participates in further aggravating the disease.

The goal of this review is to 1) present a general overview of the IFN- pathway, 2) explore how the IFN-I pathway is induced, focusing on endogenous stress activators such as host-derived self-nucleic acids, and 3) outline how kidney specific cells are affected by these cytokines.

2. IFN-I Induction In Sterile Inflammation

An early immune response to foreign pathogens, such as viral nucleic acids, is the activation of intracellular pattern-recognition receptors (PRRs), leading to the induction of IFN-I expression. These responses can also be triggered by endogenous danger signals, including self-nucleic acids detected

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in the cytosol[22] by proteosome dysfunction leading to proteotoxic stress,[23] and by elevated cellular oxidative stress.[24] In physiological homeostatic conditions, mechanisms are in place to avoid aberrant PRR activation by self-nucleic acids. This is achieved by their quick degradation using DNA and RNA nucleases, protective epigenetic and posttranscriptional modifications, and by physically compartmentalizing nucleic acids to the nucleus and mitochondria. Certain stress-inducing conditions, however, can cause nucleic acid damage and drive their release into the cytoplasm. These conditions include the presence of xenobiotics, metabolic stress, elevated reactive oxygen species (ROS), and the unfolded protein response (UPR).[25,26]

In many cases of CKD, including several types of GN, patient biopsies show evidence of DNA damage.[27] In a retrospective study using human biopsy samples, Ott *et al.* observed DNA fragmentation across a spectrum of different types of GN.[28] More specifically, it was shown in mice that double stranded DNA breaks in podocytes caused by the endonuclease I-Ppol correlated with proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis.[29] This study also identified portions of the immune system as being responsible for the development of disease. Similarly, Dhillon *et al.* show that the expression of DNA damage-causing transposable elements (TE) and endogenous retroviruses (ERVs) could be detected in diseased samples from human patients and mouse models of renal fibrosis.[30] Interestingly, Dhillon *et al.* link the expression of ERVs to the IFN-I pathway. In support of these findings, De Cecco *et al.* show an association between retrotransposable elements and IFN-I pathway signaling trough the detection of cytoplasmic cDNA.[31] As previously mentioned, the first response is to degrade nucleic acids. Mechanisms to guard against immune activation caused by self-nucleic acid detection exist in the kidney. For example, case-study data report that the nucleases TREX1 and RNaseH2[15,32] are key enzymes involved in cytoplasmic nucleic acid degradation and when mutated have been linked to kidney-predominant thrombotic microangiopathy (TMA) and Aicardi-Goutières syndrome,[15,32] respectively.

The second response involves the immune system, and more specifically the activation of intracellular PRRs. There is a vast variety of PRRs able to recognize nucleic acids, among these are the cGAS-like receptors (cGLR), RIG-I-like receptors (RLRs), and toll-like receptors (TLRs).[22] Table 1 provides a general summary of the distinct types of intracellular PRRs and their cognate ligands.

Table 1. Intracellular pattern recognition receptors (PRRs) involved in IFN-I induction.

Family	Name	Ligands	Cell distribution	Source of endogenous activators
cGLRs	cGAS	dsDNA	Cytoplasm	• Nuclear DNA • Mitochondrial DNA
RLRs	RIG-I	dsRNA	Cytoplasm	• Mitochondrial dsRNA • Transposable elements (TE) in form of Alu RNA, RNA:DNA duplex, • Misedited RNA with secondary structures
	MDA5	dsRNA	Cytoplasm	
TLRs	TLR3	dsRNA	Endosomes	• Extracellular nucleic acids from damaged bystander cells
	TLR7	ssRNA		
	TLR8	ssRNA		
	TLR9	dsDNA		

Although different nucleic acid receptors activate different signal transduction pathways, most converge at the nexus of activating TANK-binding kinase 1 (TBK1) and, in turn, the phosphorylation of interferon regulatory factor (IRF)3 and IRF7, transcription factors that allow IFN-I expression. Once IFN-Is are released, they act in an autocrine/paracrine manner through their recognition by IFN-I receptors (IFNAR1/2). IFN-I ligand-receptor binding activates a signal transduction pathway that allows for the formation of an interferon stimulated gene factor 3 (ISGF3) complex, leading to the expression of hundreds of interferon-stimulated genes (ISGs).[33,34]

Figure 1 provides a graphical representation of the main molecular interactions which occur to initiate and propagate signaling through the IFN-I pathway. The remainder of this review will

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highlight certain aspects of these interactions as they relate to the available data concerning glomerulonephritis and other prominent kidney diseases.

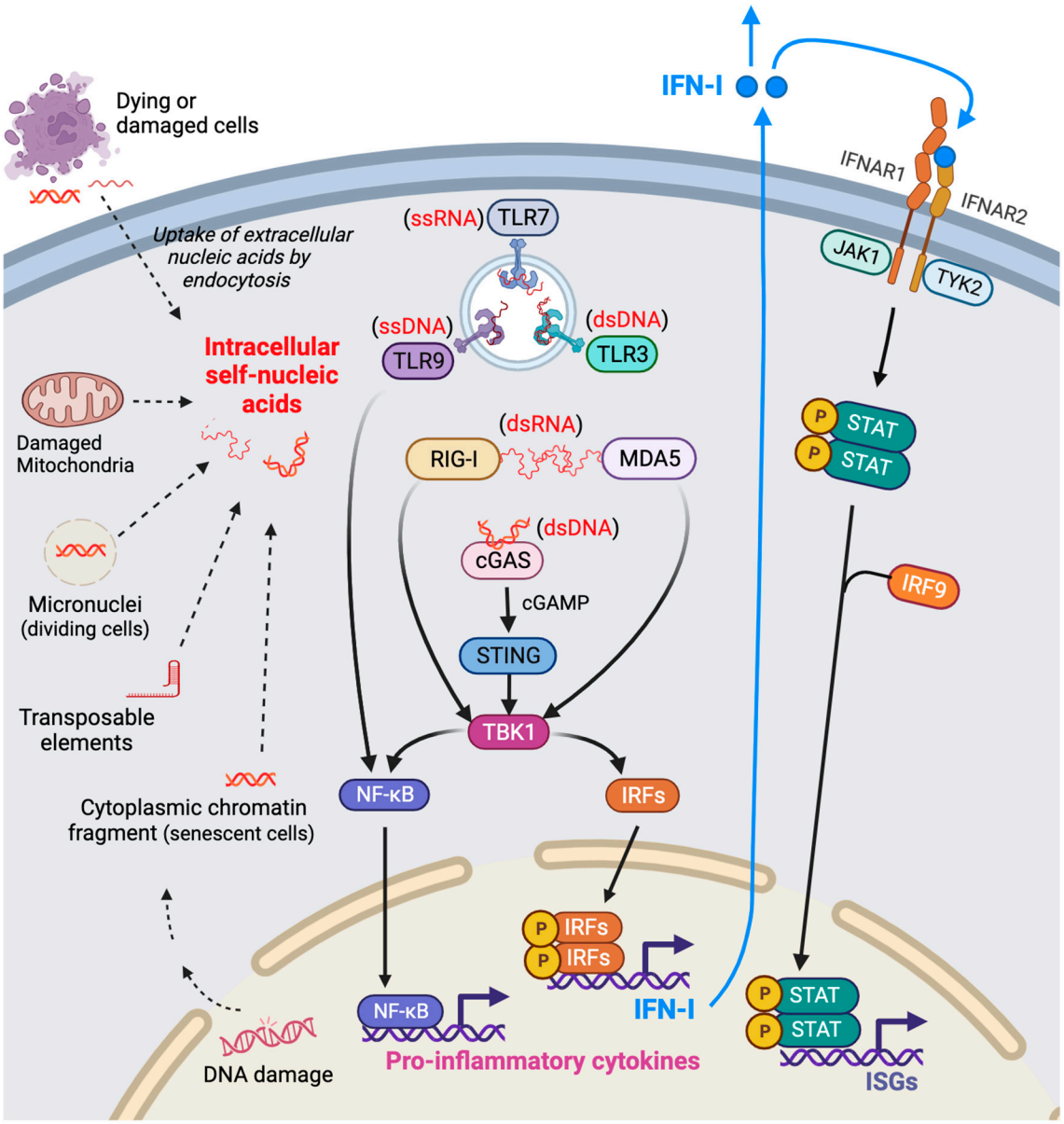

Figure 1. Origins and intracellular sensors of self-nucleic acids promoting the IFN-I signaling pathway. During cellular stresses, self-derived nucleic acids (DNA or RNA) are released from damaged mitochondria and the nucleus, consequently accumulating in the cytosol. In addition, extracellular nucleic acids liberated from neighboring dying or damaged cells are internalized by endocytosis. These intracellular (cytosolic or endosomal) nucleic-acids are recognized by diverse intracellular nucleic acid sensors, triggering the activation of the signaling pathways that produce IFN-I and proinflammatory cytokines. Specifically, sensors for endosomal nucleic acids include TLR3 (for double-stranded DNA), TLR7 (for single-stranded RNA), and TLR9 (for single-stranded DNA). Cytosolic double-stranded RNA is detected by RIG-I or MDA5, while cytosolic double-stranded DNA is recognized by members of the cGAS-STING pathway. Activation of these intracellular nucleic acid sensors stimulates TBK1 activation, prompting the translocation of IRFs and NF-kB into the nucleus. There, they orchestrate the expression of IFN-I and proinflammatory cytokine genes. Subsequently, binding of IFN-I to IFNAR1/IFNAR2 triggers the activation of the JAK-STAT pathway, culminating in the induction of ISGs. This cascade of events illustrates the intricate molecular mechanisms involved in the recognition of self-nucleic acids, the subsequent activation of signaling pathways leading to the expression and secretion of IFN-I and proinflammatory cytokines, and the subsequent

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induction of ISGs via the JAK-STAT pathway. Created with BioRender.com. IFN-I, type I interferons; TLR, Toll-like receptor; RIG-I, retinoic acid-inducible gene I; MDA5, melanoma differentiation-associated protein 5; cGAS, cyclic GMP-AMP (cGAMP) synthase; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; IFNAR, type I interferon receptor; ISGs, interferon-stimulated genes.

3. cGAS/STING Activation In The Kidney

cGAS and its analogs, cGAS-like receptors (cGLR), are evolutionary conserved receptors in metazoans that detect cytosolic double stranded (ds) DNA.[35] Once these receptors recognize their ligands, they have the ability to enzymatically convert triphosphate nucleotides into signaling linear or cyclic dinucleotides. For example, cGAS can generate 2′3′-cGAMP, a second messenger that binds and activates STING in the endoplasmic reticulum (ER).[36-38] STING then oligomerizes and translocates to the Golgi, leading to the activation of TBK1 and IRF3, which in turn translocates to the nucleus and induces IFN-I expression.

Primarily due to its cellular localization and inability to discriminate between host and foreign dsDNA, the cGAS/STING system is among the first mechanisms to respond to nuclear and mitochondrially (mt)-derived DNA present in cytoplasm. Interest in the cGAS/STING pathway has recently increased within the field of nephrology with the discovery that key glomerular cells express components of the pathway at the early stage of kidney dysfunction. Zang *et al.* show that podocyte injury can be triggered in a mouse model of diabetic kidney disease (DKD) through lipotoxicity-induced mitochondrial damage and mtDNA leakage leading to the activation of the cGAS/STING pathway.[3] Mitrofanova *et al.* observed similar results in cultured human and mouse podocytes as well as in mouse models of DKD and Alport syndrome that were exposed to a STING-specific agonist.[2] In an attempt to identify molecular pathways responsible for apolipoprotein 1 (APOL1)-induced kidney dysfunction, Wu *et al.* identified the expression of STING as an early event leading to podocyte cytotoxicity and cell death.[39] In all cases, pharmacologic inhibition or genetic deletion of the STING pathway reduced glomerular damage in these mouse models of disease. A more detailed exploration of the involvement of the cGAS/STING pathway in the progression to Lupus Nephritis End Stage Renal Disease (LN-ESRD) using cultured human podocytes was recently reported. Davis *et al.* show that if podocytes are treated with nucleosome-associated dsDNA fragments, a common marker found in the blood of Lupus patients, IFNγ-inducible protein 16 (IFI16) triggers the expression of both IFNβ and APOL1 and the activation of the cGAS/STING pathway.[40] More directly, this group shows that exposing podocytes to IFNβ promotes the expression of IFI16, suggesting a positive feedback loop between dsDNA recognition and cGAS/STING pathway activation. Additional evidence implicating the cGAS/STING pathway in lupus-mediated podocyte damage was provided by Li *et al.*, who treated mouse podocytes with LN patient serum. It was shown that in these podocytes, the cGAS/STING pathway gets activated, IFN-I expression is stimulated, and ER stress is induced leading to apoptosis.[41] Podocytes from diabetic mice also show increased levels of cGAS and STING as well as activation of TBK1. Palmitic acid, a fatty acid associated with DKD, can elicit mitochondrial damage and leakage of mtDNA into the cytoplasm to activate the STING pathway. As a result, several inflammatory markers are induced and levels of apoptosis increase. Interestingly, IFN-I expression does not seem to be elevated in this mouse model.[3]

The bulk of research linking the activation of the cGAS/STING pathway to glomerular disease has focused on the podocyte, however, some evidence exists showing that other cells within glomerulus are affected by this pathway and could contribute to renal dysfunction. There are several reports that implicate the STING pathway, either directly or indirectly, in glomerular endothelial cell damage. In diabetic mice, Qi *et al.* present evidence that mtDNA contributes to glomerular damage indirectly through GECs by inducing podocyte loss leading to proteinuria,[42] while Caselena *et al.* show that GECs display severe mitochondrial damage when treated with high glucose media.[43] Mitochondrial dysfunction is also seen in GECs from a transgenic mouse model of FSGS. This study shows the crosstalk between glomerular cells where the activation of tumor growth factor β receptor 1(TGFβR1) in podocytes is associated with an increase in mitochondrial stress and release of mtDNA

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in GECs, leading to a subsequent loss of podocytes.[44] More indirect evidence comes from studies using mice expressing an APOL1 renal risk variants (RSK). The endothelial cells of these mice display high levels of mitophagy with mtDNA leakage into the cytoplasm and IFN-I pathway activation.[45] Although mostly indirect, there is evidence that the cGAS/STING pathway gets activated in GECs and that this activation leads to glomerular damage. It is interesting to note that in 2009, Hagele *et al.* reported that GECs could be activated by dsDNA in a TLR-independent manner, showing increased IFN-I pathway expression;[46] the report being published in years close to the cGAS/STING pathway was discovered.[47,48]

Activation of the cGAS/STING pathway in mesangial cells (MCs) is still somewhat unexplored. There is some evidence, however, that mitochondrial damage leading to leakage of mtDNA occurs in cultured human mesangial cells (HMCs). This group showed that culturing HMCs with galactose deficient IgA from IgAN patients leads to mitochondrial damage due to the reduction in peroxisome proliferator-activated receptor alpha (PPARα) expression.[49] Curiously, MCs grown in hyperglycemic conditions also shown markers of mitochondrial damage, suggesting that leakage of mtDNA and the subsequent activation of the cGAS/STING pathway could also be operating in some instances of DN.

It should be including here that there are other cell types not typically associated with the glomerulus that could also contribute to glomerular injury through the STING pathway. As an example, plasmacytoid dendritic cells (pDCs) are a special type of immune cell characterized, in part, by their high expression of IFN-I. Indeed, Deng *et al.* show that pDCs rapidly infiltrate the kidney after AKI and produce IFNα, leading to kidney damage.[50] These cells have also been implicated in lupus nephritis,[51,52] where it has been reported that STING activation promotes the maturation of pDCs with their subsequent participation in glomerular injury.[53] However, it must be emphasized that it is still not clear if pDCs' contribution to renal disease is through their involvement systemically or at the level of the kidney.

4. RLRs Activation in The Kidney

Melanoma differentiation-associated gene 5 (MDA5) and retinoic acid inducible gene I (RIG-I), are two RLR family members which reside in the cytoplasm and act as receptors for dsRNA, and in a manner similar to cGAS, activate the mitochondrial antiviral-signaling protein (MAVS), TBK1, and IRF3 to elicit IFN-I expression. Although cytoplasmic endogenous dsRNA is a less common indicator of cellular stress as compared to cytoplasmic dsDNA, sources include ERE, mt-dsRNA and other secondary RNA structures that arise from epigenetic alterations, mitochondrial damage, or defects in RNA processing.[54-59] Murine models of autoimmune disease have been instrumental in showing that mt-dsRNA plays a relevant role in driving sterile inflammation through IFN-I pathway activation.[60,61]

The activation of RLRs has been documented in a variety of kidney diseases, both in human patient samples and animal models of AKI and several different types of GN. A number of studies have shown that tubule-specific damage is linked to active RLRs. For example, Zhu *et al.* showed that renal tubule injury in mice caused by ischemia-reperfusion-injury (IRI) or unilateral ureteral obstruction (UUO) was closely tied to an accumulation of mt-dsRNA.[62] Similar results were reported by Doke *et al.* also using the murine IRI method.[63] Exploring the mechanisms responsible for AKI caused by Crush Syndrome (CS), Wang *et al.* utilized rats and were able to show a significant increase in RIG-I associated signaling.[64] A study using whole exome sequencing on patients with LN identified a gain-of-function mutation in RIG-I leading to its constitutive activation.[65] RLR activation and associated inflammation was observed in kidney samples from CKD patients showing an increase in ERE expression.[30]

Cells within the glomerulus also express the major sensors for dsRNA and can activate the associated immune signaling pathways. Yamashita *et al*. used cultured human and mouse podocytes to show that regardless of the source of dsRNA, be it from mitochondria, ERE or secondary structures spontaneously formed, RLR activation in podocytes leads to the expression of IFN-I, IL-6, and cytoskeleton alterations,[66] the latter being instrumental in causing effacement and eventual cell loss.[67] The remaining bulk of evidence linking RLRs to podocyte dysfunction comes from investigations

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into the mechanisms whereby APOL1 causes kidney damage. Fang *et al.* used long-term injections of recombinant APOL1 in mice and observed increased expression of RIG-I in podocytes. Similar findings were seen in cultured human podocytes engineered to express APOL1 RRV. Knock-down of RIG-I, either using siRNA against RIG-I in *in vitro* experiments or AAV-shRIG-I in *in vivo* mouse experiments, blunted the expression of several pro-inflammatory genes and attenuated podocyte and glomerular damage, respectively.[68] One caveat emerging from this study is that it remains unclear what the direct activator of RIG-I is, and if RIG-I drives the expression of IFN-I along with other proinflammatory genes. Further confounding the involvement of RLRS in APOL1-mediated podocyte damage is the discovery that APOL1 mRNA itself possesses structural characteristics that allows for the formation of dsRNA secondary structures,[69] which indeed have been shown to be recognized by MDA5 in podocytes.[70] However, as just mentioned, it is yet to be determined if these APOL1 secondary structures can be also recognized by RIG-I.

There is a dearth of evidence for the expression of RLRs in other glomerular cells. Results from *in vitro* experiments support the notion that RLRs can contribute to glomerular injury. Hagele *et al.* used a synthetic agonist of RLR to treat cultured murine GECs. These experiments revealed that there is an increase in the expression of IFN-I, IL-6, and intracellular adhesion molecule 1 (ICAM-I). It can be extrapolated that these changes in GEC could lead to increased albumin permeability.[71] The same synthetic agonist was used to *in vitro* treat human mesangial cells. The result was an increase in the expression of RIG-I chemokine ligand 5 (CCL5) and the C-X-C motif chemokine ligand 10 (CXCL10).[72,73] Taken together, these results suggest that if GECs and MCs are under stress and are exposed to RLR ligands, they are able to promote inflammation favoring chemotaxis and the adhesion of leukocytes. However, since the synthetic ligand for RLRs can also activate TLR3, signaling through TLR3-mediated pathways cannot be ruled out.

5. TLRs Activation in The Kidney

TLRs are comprised of a large family of PRRs that are expressed on the plasma membrane and intracellularly, the latter location being activated by nucleic acids and highly associated with sterile inflammation.[74] Endosomally-restricted TLRs include: TLR3 (which recognizes dsRNA), TLR7/8 (which recognizes ssRNA), and TLR9 (which recognizes dsDNA). It must be highlighted that intracellular TLRs cannot participate in the first response to self-derived nucleic acids in the cytoplasm as the other RLRs mentioned above can since they are physically sequestered within endosomes. Instead, these TLRs are only activated in the event that extracellular DNA or RNA from damaged or dead bystander cells has been endocytosed and the nucleic acid-containing endosome fuses with an acidified endosome containing the TLRs.[75] Indeed, there are several reports in the literature where extracellular mRNA or mitochondrial RNA derived from damaged cells has been engulfed by neighboring cells resulting in TLR3 activation and sterile inflammation.[60,76,77] Highlighting a route for entry for nucleic acids, Bertheloot *et al.* show that RNA released from damaged cells can be detected by the receptor for advanced glycation-end products (RAGE) to elicit their internalization and activate TLR7 and TLR8. [78] It is thought that a similar mechanism exists for the detection and internalization of mtDNA from damaged mitochondria, leading the activation of TLR9.[79] Extracellular nucleic acids are shielded from degradation if they are bound to other molecules. These shielding molecules include antibodies, antimicrobial peptides, neutrophil extracellular traps (NETS), and microvesicles; all of which have been detected in patients with lupus.[80-83] Once formed, these nucleic acid-protein complexes can be taken up through endocytosis by specialized cells, including macrophages and dendritic cells, but also by podocytes, MCs and GECs.[84-86] However, we should consider that the activation of this class of TLRs could be the consequence of previous IFN-I or other pathway activation and/or as a result of actions carried out by any number of immune cell types, rather than directly by cells within the glomerulus.

In the context of CKD and GN, the function of TLRs has been extensively reviewed in previous publications.[87,88] Therefore, this review will narrowly focus on the involvement of TLR signaling in podocytes, GECs, and MCs.

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Experiments using cultured human podocytes confirm that these cells highly express TLR3 and upon its activation, were shown to induce the expression of IFNβ, several chemokines (IL-6, MCP-1, CCL5), the costimulatory molecule CD80, and APOL1, but not IFNα.[89-91] TLR7 agonists have been widely used to generate mouse models of lupus which display varying degrees of podocyte injury; however, podocyte damage can be traced to the action of other cell types, including those that belong to the immune system.[92,93] A more direct involvement of TLR8 in podocyte damage has been seen in mice models of lupus and in rodents that have undergone UUO. In these experimental models, TLR8 expression is increased in podocytes and is associated with the elevation of micro-RNA 21 (miR21),[94,95] an endogenous ligand for TLR8.[96] Interestingly, it has been reported that miR21 is increased in the urine and renal tissue of CKD patients, including those diagnosed with DN and IgAN.[97-99] In a rat model of nephrotic syndrome and in a mouse model of autoimmune GN, TLR9 expression is increased and associated with podocyte injury.[100,101] Moreover, mtDNA and/or dsDNA seems to be the ligand for this receptor in podocytes, inducing their apoptosis *in vitro*. Seemingly contradictory results were reported by Bossaller *et al.*, who show that TLR9 deficiency aggravates LN induced by TLR7 agonists in mice,[102] suggesting a role for TLR9 as a negative regulator to limit glomerular damage.

Only the involvement of TLR3 in GECs has been reported. A number of publications originating from the same group using cultured human cells, show that the *in vitro* activation of TLR3 elicits the expression IFN-I, RLRs, chemokines and plasminogen activator inhibitor-1 (PAI-1), a negative regulator of fibrinolysis.[103-108] Taken together, this body of work suggests that the activation of TLR3 in GECs may contribute to inflammatory and fibrotic responses, events that could also be a result of a secondary response to the IFN-I pathway signaling.

Similar to reports for GECs, much of the work exploring the action of TLRs in MCs was accomplished using cultured human mesangial cells. These studies show that the in vitro activation of TLR3 in MCs results in the expression of several different chemokines, adhesion molecules and matrix metalloproteinase 9 (MMP9).[109-112] It is worth mentioning that MMP9 is an enzyme relevant to the inflammatory response and is detected in the biopsies of patients diagnosed with active and chronic GN,[113] as well as in the urine of patients with MN.[114] A report by Shen *et al*. revealed that TLR9 was significantly elevated in MCs grown *in vitro* under hyperglycemic conditions and that knockdown of TLR9 under these same conditions reduces the expression of several inflammatory markers, and the incidence of apoptosis.[115] In the same study using a mouse model of diabetes, silencing TLR9 reduced glomerular matrix cell expansion.

Taken together, these various reports suggest that under conditions that result in cellular stress, nucleic acids may be released into the extracellular space where they can be taken up by any number of different cells, including cells within the glomerulus. This, in turn, results in the activation of the IFN-I pathway and may include other signal pathways such those that induce nuclear factor κB (NF-κB) activation. The end result is to prolong and propagate sterile inflammation in the kidney.

6. Effect of IFN-I in Glomerular Cells

The activation of intracellular PRRs leads to the synthesis of IFN-I. The term 'IFN' encompasses a group of cytokines divided into three family types referred as type I, type II (also named g), and type III (also named l). Interferons were initially discovered for their essential role in the anti-viral and anti-cancer immune response, and more recently, for their participation in autoimmune and autoinflammatory disorders.[116-119] Among the IFN-I family members, several subtypes have been described in mammals and include a, b, d, e, k, t, and w. In humans, IFN-I contains 13 isoforms of IFNα and one isoform of IFNb, and are primarily recognized by their biological function.[116,120]

IFN-I are recognized by a heterodimeric receptor composed by interferon a and b receptor subunits 1 and 2 (IFNAR1 and IFNAR2). Although both subunits are necessary for IFN-recognition, the signal transduction is initiated by IFNAR1. Attached to the cytoplasmic tail of IFNAR1 is tyrosine kinase 2 (TYK2) which is initially activated, followed by the phosphorylation of Janus kinase 1 (JAK1), the phosphorylation of signal transduction and activator of transcription (STAT)1 and STAT2, which are associated with the cytoplasmic tail of IFNAR2. Next is the formation of ISGF3, a heterotrimeric

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transcriptional factor made up of STAT1, STAT2, and IRF9 which functions to promote the expression of interferon signature genes (ISGs). Cellular stress, including ribosomal stress, drives crosstalk between the above-mentioned canonical IFN-I pathway and other signaling pathways to boost the expression of hundreds of ISGs. The end result is the alteration of several cellular processes such as transcription, translation and metabolism.[121-123]

Recently, Manoharan *et al.* report that the cytokine tissue factor (TF) exhibits substantial homology with IFNAR2 and can physically interact with IFNAR1 to restrict its activation in glomerular cells under steady state conditions. Moreover, TF deletion in podocytes promotes IFN-I pathway activation and the development of GN. Interestingly, biopsies from patients diagnosed with different forms of GN show a reduction in TF levels within the glomerulus.[124]

The effects of IFNα signaling in podocytes includes the induction of autophagy and the inhibition of the mammalian target of rapamycin complex 1 (mTORC1) pathway.[125] These observations provide an additional link between IFN-I signaling and the development of GN since the loss of mTORC1 in podocytes causes proteinuria associated with glomerular damage.[126] Furthermore, IFNβ induces the expression of APOL1[91] and apoptosis in mature podocytes, along with the reduction of nephrin expression during podocyte differentiation *in vitro*.[20] Li *et al.* also show that IFN-I activation leads to the induction of viperin (also known as RSAD2) a common ISG reported to be a negative regulator of podocyte differentiation.[127] Similar effects have been described in other kidney-specific cells, such as parietal epithelial cells (PECs) and tubule epithelial cells (TECs), where IFN-I signaling disrupts the cell cycle and promote cell death.[20,128] It is becoming clear that elevated IFN-I expression in podocytes can drive autophagy, impair cellular differentiation, and promote podocyte apoptosis, events that can exacerbate glomerular disease. On the other hand, Lee *et al.* showed that by targeting IFNAR1/2 signals using bariticinib, a JAK inhibitor, podocyte damage in a mouse LN model could be reduced, along with a general reduction in inflammation pointing to a systemic effect of the inhibitor.[129]

There is little direct evidence of what effect IFN-I has on glomerular endothelial cells. A series of publications from Hiroshi Tanaka's group using cultured human GECs identified a set of ISGs with the potential to be used as biomarkers for LN.[103,130,131] Instead, we might draw on work accomplished using other types of endothelial cells to extrapolate IFN-I signaling effects on GECs. For example, work using brain, liver, and lung endothelial cells indicates that IFN-I cytokines, mainly IFNβ, affects tight junctions through the down-regulation of VE-cadherin, reducing the synthesis of nitric oxide (NO), the induction of PAI-1 secretion, and the reduction in caveolin 1 expression.[132-134] Combined, these effects could promote endothelial permeability and stiffness. Additionally, interferon-stimulated gene 15 (ISG15), one of the byproducts of IFNAR1/2 activation, has been linked to the development of vascular stiffness in cases of hypertension.[135]

In mesangial cells, it has been demonstrated that IFNβ promotes the expression of IL-6 and participates in regulating proliferation.[136] Using primary human mesangial cells, Zhang *et al.* discovered a feed-forward regulatory pathway where IFN-I stimulates the expression of miR744, which boosts the activation of JAK1/STAT1/STAT2 to promote the expression of several chemokines, including CXCL10.[137] More recently, Gao *et al.* show that CXCL10 can stimulate mesangial cell proliferation and migration *in vitro,* and participates in mesangial cell expansion in a mouse model of glomerulonephritis.[138]

7. Concluding Remarks and Future Perspectives

The IFN-I pathway has been extensively studied as part of the innate immune system's response against viral infections. This pathway originates with the recognition of viral nucleic acids by intracellular PRRs, followed by the transduction of signals that induce IFN-I production and release, and terminates with the recognition of IFN-I by specific receptors to induce the expression of genes that reduces transcription and translation rates, as well as alter nucleotide acid and lipid metabolism to block the viral replication cycle.[139] However, the function of this pathway in sterile inflammation is not yet fully understood. With the description of interferonopathies more than a decade ago, much attention turned to focus on the relationship between these cytokines and autoinflammatory

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effects.[119,140] Soon to follow was the discovery and description of a new cytoplasmic nucleic acid receptor system, cGAS/STING which also was linked to IFN-I expression.[47,48] Henceforth, new light was shed on how endogenous stress, through self-nucleic acid recognition, drives sterile inflammation via cGAS/STING and other PRR signaling pathways.

Although there is ample evidence supporting the hypothesis that blocking intracellular receptors blunts sterile inflammation, there still exists a substantial gap in knowledge regarding the exact mechanisms responsible. This is especially true if the protective effects are due to limiting IFN-I expression or if other pathways are the primary stimulators of the inflammation since these intracellular receptors can also participate in crosstalk with other signaling pathways that activate NF-kB.

This review explored and described the endogenous activators of intracellular PRRs that drive IFN-I expression, as well the specific effects these cytokines have on kidney and glomerular cells that, when considered together, could indicate a direct involvement of this pathway in GN progression. Figure 2 summarizes the key findings of this review with respect to the effect of the IFN-I pathway on glomerular health. Many questions, however, remain unanswered. For example, is it possible that IFN-I pathway activation is merely an indirect reaction to an initial insult rather than the main cause of GN? Since GN is a group of diseases with each group displaying complex pathophysiology, it may prove difficult to accurately uncover the responsible mechanisms for each since *in vivo* and *in vitro* models seldom fully resemble the pathology displayed by human patients. Nonetheless, and despite the complexity of the molecular mechanisms involved, it is clear that the IFN-I pathway is participating in the development and progression of GN. The evidence of IFN-I pathway function in glomerular cells presents an opportunity to explore the potential exploitation of this pathway for therapeutic purposes. For example, manipulation of the IFN-I pathway and related cytokines could boost the production of antibodies for vaccine development[141] directed at autoimmune driven GN. Another avenue to explore is the effect that IFN-I has on the cellular metabolism of glomerular cells, including glycolysis, oxidative phosphorylation, and lipid synthesis, as has been reported for macrophages and T cells.[142-144] The relationship the IFN-I pathway has with other cellular stress responses, such as the activation of inflammasomes and the unfolded protein response (UPR)[23,145] could be investigated further, specifically in glomerular cells to inform new treatment options. Finally, new insights into renal fibrosis, glomerular cell senescence, and aging could be gained by exploring IFN-I pathway crosstalk with tumor growth factor β (TGF-β) pathway,[146] DNA damage and epigenetic changes,[147] or inflammaging process,[148] respectively.

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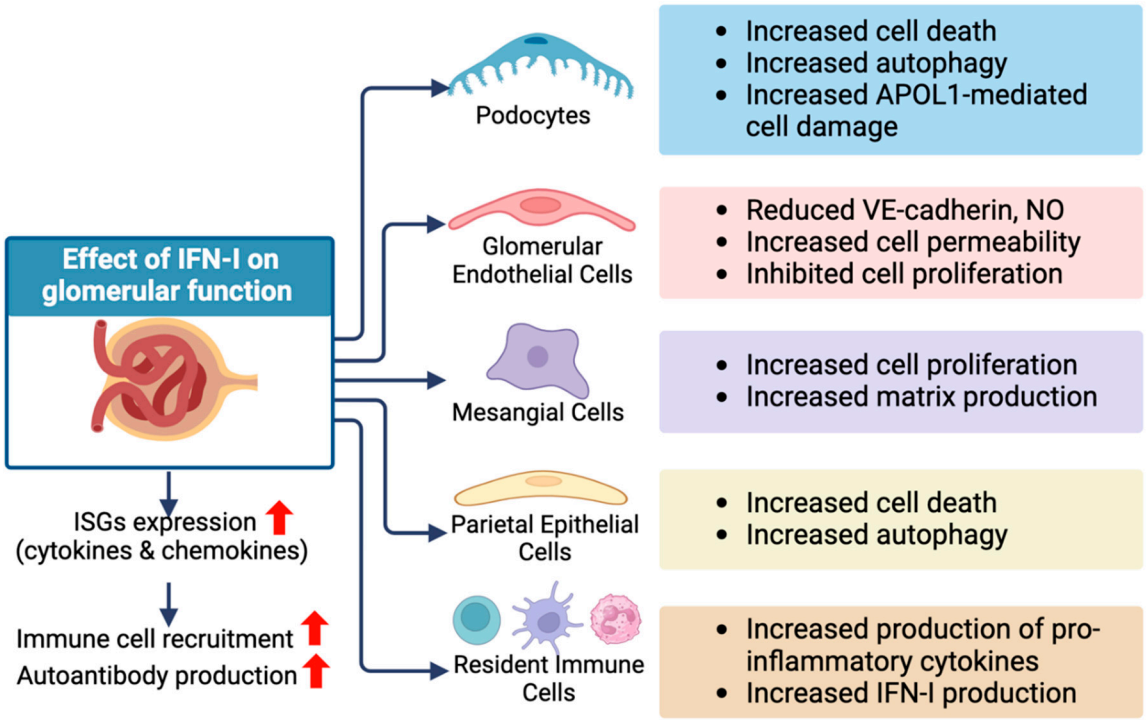

Figure 2. The potential deleterious effects of IFN-I on different glomerular cell types. IFN-I can exhibit direct influence on diverse cell types within the glomerular compartment, altering their cellular functions. In addition, increased IFN-I levels induce the expression of interferon-stimulated genes (ISGs). This could intensify inflammatory processes by recruiting inflammatory immune cells to the kidney and increasing the production of autoantibodies. Created with BioRender.com. APOL-1, Apolipoprotein L1; VE-cadherin, Vascular endothelial-cadherin; NO, Nitric oxide.

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