Chapter 3

Toll-Like Receptor 3 and Retinoic Acid-Inducible Gene-I Implicated to the Pathogenesis of Autoimmune Renal Diseases

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Additional information is available at the end of the chapter

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1. Introduction

The innate and adaptive immune systems have been reported to play an important role in the pathogenesis of glomerular diseases. Since viral infection may sometimes trigger the development of inflammatory renal disease or the worsening of pre-existing renal disease, recent studies have focused on the involvement of toll-like receptors (TLRs) and their signaling pathways in the inflammatory processes of glomerular cells [1]. Recognition of the molecular pattern of a pathogen, which is distinguishable from host molecules, is important in innate immunity, and TLRs are specialized in the pattern recognition of pathogen molecules. The activation of TLRs and their downstream immune responses can be induced not only by infectious pathogens, but also by non-infectious stimulation, such as endogenous ligands, and this mechanism may be involved in the pathogenesis of autoimmune renal diseases [1-3]. Viral double-stranded RNA (dsRNA) can activate not only TLR3 located in intracellular endosomes, but also retinoic acid-inducible gene-I (RIG-I)-like helicases receptors located in the cytosol [4]. RIG-I and melanoma differentiation-associated gene-5 (MDA5) are members of RNA helicase family in the cytosol, and both act as pathogen recognition receptors [5]. Therefore, RIG-I and MDA5 may also involved in the pathogenesis of autoimmune renal diseases [6-8].

Recent studies revealed the expressions of TLRs in resident renal cells, suggesting the involvement of the TLR signaling pathway in the pathogenesis of glomerular diseases [1-3]. Once presumptive antigenic ligands bind to TLRs, the activation of transcriptional factors, such as interferon regulatory factors (IRF) and nuclear factor kappa B (NF-κB) is induced through intracellular signaling cascade activation. The activation results in the

release of adhesion molecules, cytokines and chemokines, which play a pivotal role in the innate and adaptive immune responses [1-3]. For example, the activation of mesangial TLR 3 during hepatitis C virus (HCV) infection contributed to chemokine/cytokine release and caused proliferation and apoptosis in the pathogenesis of HCV glomerulonephritis [9]. This is direct evidence of the involvement of TLRs in the inflammatory processes of viral-induced glomerulonephritis. It has been reported that glomerular mesangial cells (MCs) produce a wide variety of pro-inflammatory molecules that play an important role in immune and inflammatory reactions in the kidney [10]. In an experimental setting, the activation of mesangial TLR3 induced by polyinosinic-polycytidylic acid (poly IC), an authentic dsRNA, upregulated the expression of matrix metalloproteinase 9 (MMP9), plasminogen activator inhibitor type 1, and tissue plasminogen activator in human MCs. These findings suggest that viral RNA can influence the generation and degradation of the extracelluar matrix in the mesangium in ways other than through direct viral stimulation, and, subsequently, the possible development of glomerulosclerosis might occur [11, 12]. Furthermore, in another set of experimental studies using poly IC-stimulation, MCs have been reported to express functional molecules such as interleukin (IL)-6 [6], CC chemokine ligand (CCL) 2 (or monocyte-chemoattractant protein-1, MCP-1) [13], and CCL5 (or regulated on activation, normal T-cell expression and secretion, RANTES) [9].

Like TLR3, RIG-I and MDA5 may detect viral RNAs and mediate immune reactions against RNA viruses [4, 14]. It has been reported that RIG-I, and not TLR3, mediated the secretion of type I interferon (IFN) in poly IC/cationic lipid complex-treated glomerular endothelial cells [15]. In contrast, other investigators have reported that, in MCs, RIG-I was not involved in the poly IC-induced expression of IL-6 [13] or MMP9 [11], while TLR3 was involved in that system. In an interesting experiment using TLR3 signaling-deficient mice, it has been reported that MDA5, but not RIG-I, was required for signaling induced by poly IC/cationic lipid complex in murine MCs [6]. The cells transfected with poly IC/cationic lipid complex is thought to be a model of entry of RNA virus into the cytoplasm. However, the precise role of RIG-I in mesangial inflammation remains to be elucidated. Since there are few data on the role of RIG-I, and the interaction between TLR3, MDA5 and RIG-I in human glomerular diseases, we performed several experiments using cultured normal human MCs. We found that the involvement of novel RIG-I-mediated signaling pathways in mesangial inflammation in human MCs [8, 15-17]. These signaling pathways may be involved in the pathogenesis of human glomerular diseases.

2. RIG-I and lupus nephritis

We previously found significant expression of RIG-I in the glomeruli of biopsy specimens from patients with lupus nephritis, and the level of expression correlated with the severity of the acute inflammatory lesions (Figure 1.) [18].

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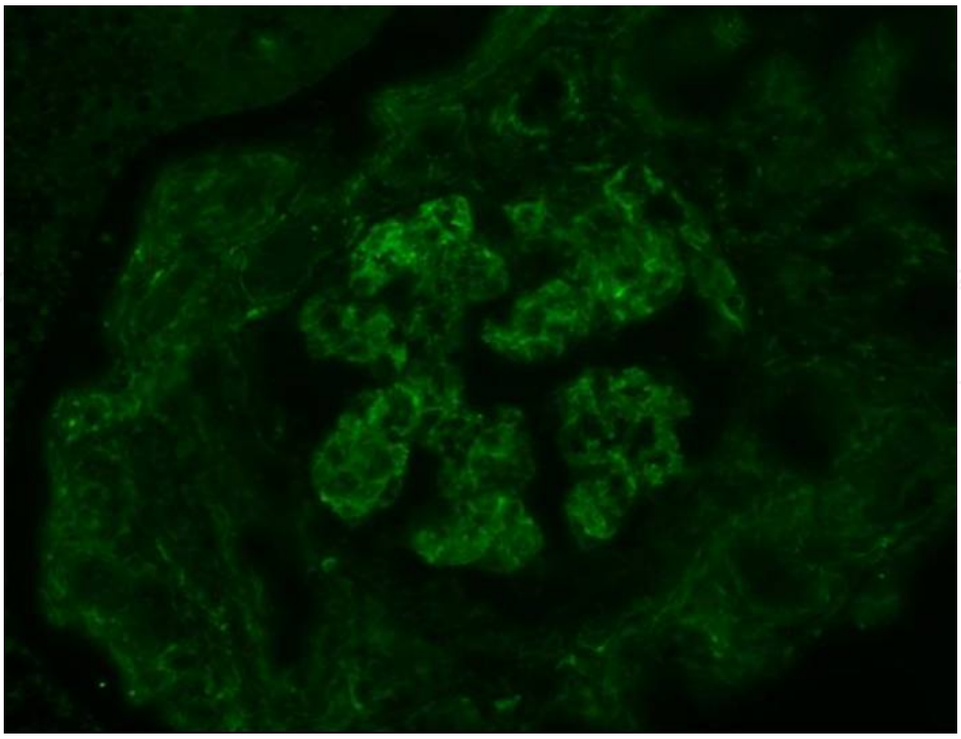

Figure 1. Glomerular immunoreactivity for RIG-I was detectable in cases of diffuse proliferative lupus nephritis, and an intense granular pattern of immunofluorescence was observed in a mesangial area and capillary loop distribution (Suzuki et al. NDT 2007).

In addition, we found that the levels of RIG-I mRNA in the urinary sediment of patients with lupus nephritis were higher than those in patients with IgA nephropathy and controls [19]. Interestingly, repeated measurements of the mRNA expression of RIG-I in the urinary sediment of lupus patients revealed a reduction in the expression following immunosuppressive treatment [19].These findings suggest that RIG-I may be involved in the acute inflammatory process in human lupus nephritis. These clinical observations led us to conduct the following experimental studies.

In order to examine the involvement of RIG-I in lupus nephritis, we conducted experimental studies using human MCs in culture. Because Th1-derived cytokines are known to be key mediators in the progression of lupus-associated renal injury, and IFN-γ is one of the major Th1 type cytokines with potent proinflammatory effects through the upregulation of IFN-inducible genes [3], the effects of IFN-γ on the expression of RIG-I in human MCs in culture were examined. As a result, IFN-γ treatment resulted in a concentration-dependent upregulation of the expression of RIG-I mRNA and protein in human MCs. The treatment of cells with IFN-γ also induced the expression of mRNA for both IRF1 and IRF7, which are important IFN-inducible transcriptional factors [15]. Furthermore, knockdown of RIG-I expression by small interfering RNA (siRNA) inhibited the IFN-γ-induced expression of IRF7, but not that of IRF1. In contrast, IFN-γ did not induce the expression of IFN-β, which is known to be a target gene of IRF-7, in MCs (Figure 2.) [15].

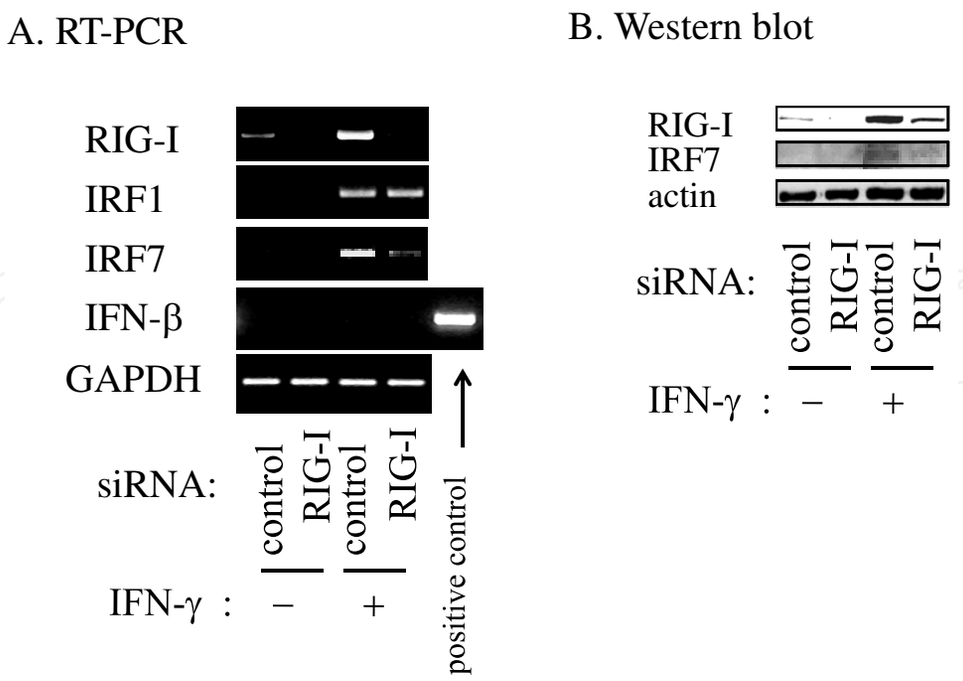

Figure 2. The cells were transfected with siRNA against RIG-I or a negative control non-silencing siRNA. At 24 h after the transfection, the cells were treated with 5 ng/ml IFN-γ for 24 h. (A) RNA was extracted from the cells and RT-PCR analyses for RIG-I, IRF1, IRF7, IFN-β and GAPDH were performed. (B) The cells were lysed and the lysates were subjected to western blot analysis for RIG-I, IRF7 and actin. (Imaizumi, et al. Lupus 2010)

Interestingly, the pretreatment of cells with dexamethasone inhibited the IFN- γ -induced expression of MCP-1 mRNA but did not affect the induction of mRNA for RIG-I or IRF7 in MCs. The induction of MCP-1 mRNA by IFN-γ was not inhibited by the knockdown of NF-κB p65, indicating that the NF−κB signaling pathway was not involved. Our results suggest selective regulation of the expression of IRFs by RIG-I in human MCs. The function of IRF7 has been well studied, mainly in dendritic cells and in mouse embryonic fibroblasts, and IRF7 is thought to be an important transcriptional factor that affects anti-viral responses by inducing the production of type I IFN [20]. However, neither IFN-γ treatment nor knockdown of RIG-I affected the expression of IFN-β in MCs. Although the functional significance of IRF7 expression in MCs remains to be elucidated, our recent observations suggest that the IFN-γ/RIG-I/IRF7 signaling pathways may be involved in the pathogenesis of lupus nephritis [15]. To date, it has been reported that TLR3, TLR4, TLR7 and TLR9 may play a role in the modulation of inflammatory processes in lupus nephritis [1, 3]. TLR7 and TLR9 recognize mammalian nucleic acids as well as bacterial DNA or viral single-stranded RNA (ssRNA), suggesting that the generation of some autoantibodies may be attributable to a possible role of TLR7 and TLR9 in selected patients with lupus nephritis [21]. Our previous clinical and experimental observations provide additional knowledge in the pathogenesis of lupus nephritis, although this remains preliminary. We believe that the involvement of the newly observed the IFN-γ/RIG-I/IRF7 pathway in MCs may contribute to mesangial inflammation, and the intervention of these signaling pathway may lead to the development of an optimal

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future therapeutic strategies in lupus nephritis. Further clinical and experimental issues remain to be examined in future studies [22].

3. TLR3 and RIG-I in human MCs

Viral dsRNA is a potent inducer of type I IFNs and the downstream molecules of the innate immune pathway. Thus, in order to evaluate the potential role of RIG-I in response to viral dsRNA in human MC, we treated the cells with poly IC, an authentic dsRNA, in the next experiment. The cells were simply treated with poly IC, not transfected using poly/cationic lipid complex, in this experiment. Treatment with poly IC is a model of cells exposed to viral dsRNA released from dying cells. Stimulation with poly IC resulted in an increase in the expression of both RIG-I mRNA and protein in a concentration- and time-dependent manner, and this was accompanied with CCL5 expression [16]. Furthermore, treatment with RIG-I siRNA significantly lowered poly IC-induced CCL5 expression. In contrast, the poly IC-induced expression of CCL2 mRNA was not affected by RIG-I siRNA (Figure 3.). Interestingly, the poly IC-induced RIG-I expression was suppressed in response to treatment with siRNA against TLR3. Furthermore, TLR3 siRNA downregulated the poly IC-induced expressions of TLR3 and IFN-β, but RIG-I siRNA did not affect the expression of either TLR3 or IFN-β. In order to examine the role of IFN-β as a potential mediator of poly IC-induced RIG-I expression, IFN-β siRNA were used. The results showed that the poly IC-induced expressions of IFN-β and RIG-I were markedly inhibited in cells transfected with IFN-β siRNA. Pretreatment of the cells with a blocking antibody against the type I IFN receptor also reduced the poly IC-induced expression of RIG-I. Moreover, pretreatment of the cells with dexamethasone reduced the poly IC-induced expression of both RIG-I and IFN-β, but this treatment had no effect on IFN-β-induced RIG-I expression [16]. Our results suggest that the expression of CCL5 was selectively regulated by RIG-I expression in human MCs, because poly IC-induced CCL5 expression was inhibited in response to the knockdown of RIG-I, while the expression of CCL2 was not affected by treatment with RIG-I siRNA. A recent report suggested that RIG-I, and not TLR3, mediated the secretion of type I IFN in poly IC/cationic lipid complex-treated glomerular endothelial cells [14]. Our findings reveal another aspect of glomerular inflammation, as the cross talk between glomerular endothelial cells and MCs may be an important factor of glomerular inflammation, and the RIG-I/CCL5 pathway in mesangial cells may contribute to glomerular inflammation, particularly after viral infection [16].

Both TLR3 and RIG-I are reported to serve as receptors for viral dsRNA. Our recent study showed that siRNA-mediated knockdown of TLR3 inhibited the poly IC-induced expression of both IFN-β and RIG-I. However, RIG-I knockdown had no effect on poly IC-induced IFN-β expression. Thus, RIG-I may function downstream to TLR3 in the signaling cascade activated by poly IC-induced expression of CCL5 in MCs [16]. In addition, the inhibitory effect of dexamethasone may depend on the suppression of IFN-β production, and not on the IFN-β-induced RIG-I expression. In this signaling pathway in MCs, TLR3 and newly synthesized IFN-β are involved in poly IC-induced RIG-I expression. Since dexamethasone had no effect

on IFN-β-induced RIG-I expression, the inhibitory effect of dexamethasone may depend on the suppression of IFN-β production. On the basis of these results, we propose the TLR3/IFN-β/RIG-I/CCL5 pathway (Figure 4.). This pathway may play an important role in immune and inflammatory reactions against viral infection in MCs. Since a viral infection may sometimes trigger the development of an inflammatory renal disease or the worsening of pre-existing renal disease, we believe that our recent findings are informative enough for the field of nephrology. Interestingly, it has been reported that tacrolimus (Tac) reduces proteinuria and mesangial alterations due to suppression of glomerular IFN-γ mRNA expression in rat models [23]. Thus, an immunosuppressant, Tac, may be a possible candidate for the intervention of these signaling pathways, although this remains to be elucidated in future studies.

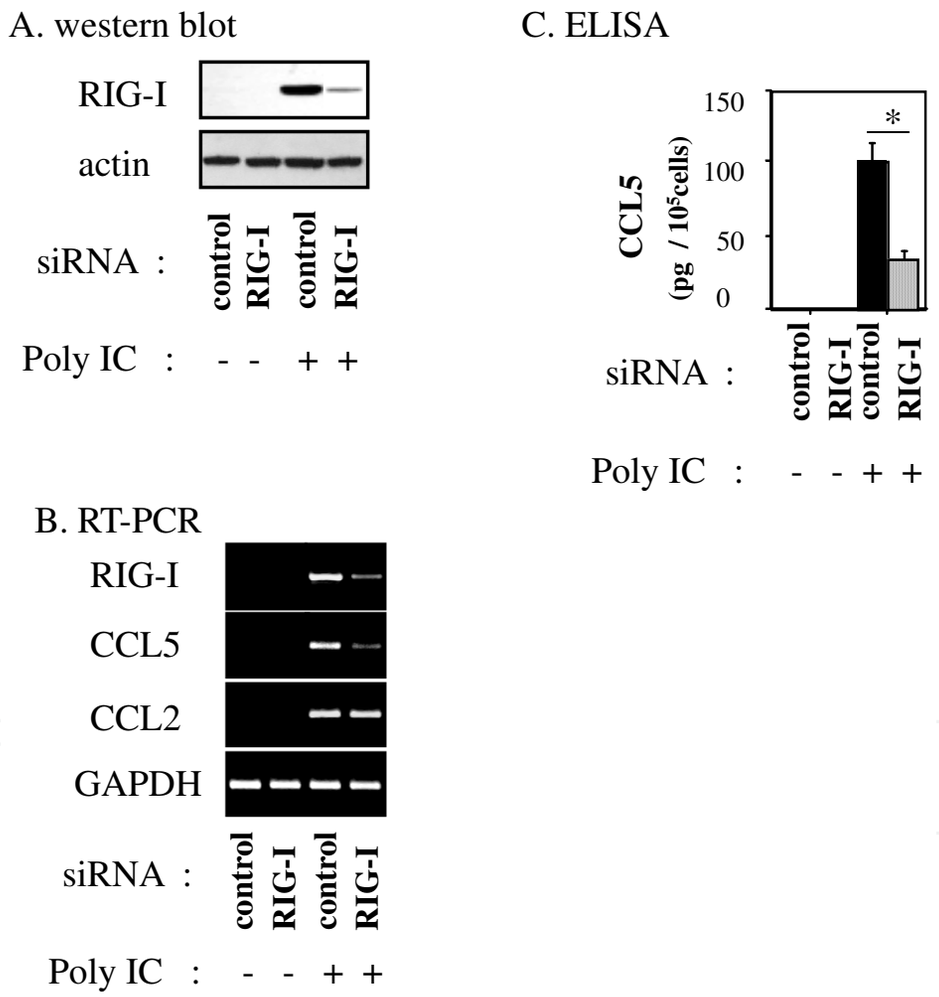

Figure 3. Knockdown of RIG-I reduces the poly IC-induced expression of CCL5 in human mesangial cells. The cells were transfected with siRNA against RIG-I or control siRNA and then stimulated with 20 mg/ml of poly IC. (A) After 24 h of poly IC treatment, the cells were lysed and western blotting for CCL5 was performed. (B) The cells were incubated for 16 h with poly IC, RNA was extracted, and RT-PCR was performed for RIG-I, CCL5, and CCL2. (C) The culture medium was collected after 24 h, and the concentration of CCL5 was determined by ELISA (n=3, *p<0.01). (Imaizumi, et al. NDT 2010)

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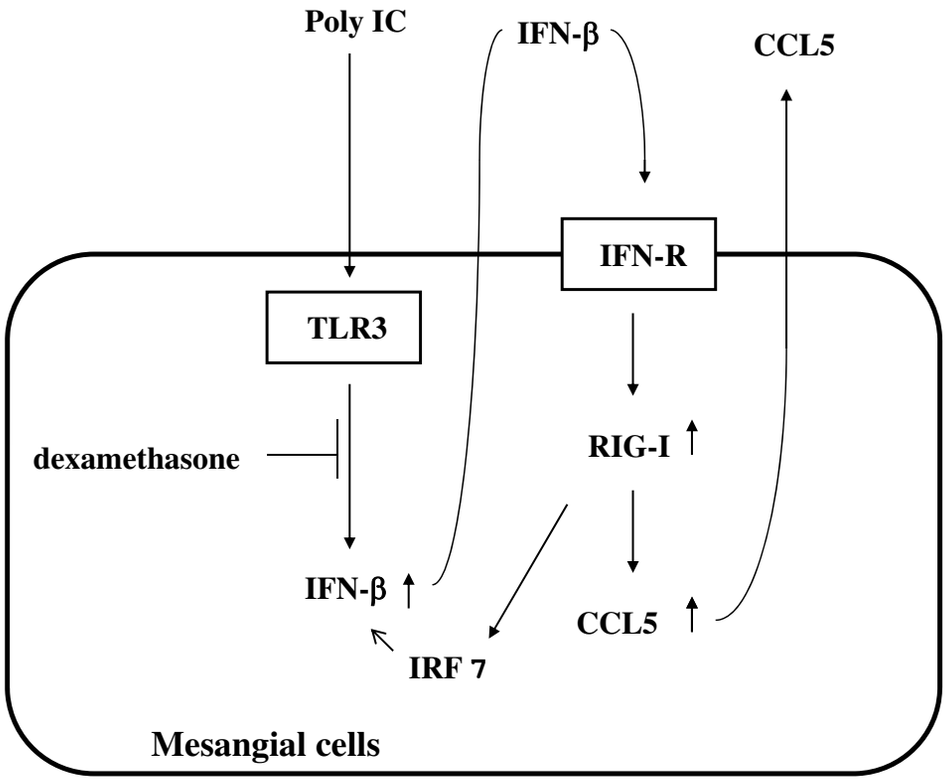

Figure 4. Proposed TLR3/IFN-β/RIG-I/CCL5 and IFN-γ/RIG-I/IRF7 signaling pathways in human mesangial cells (See the main document and Ref. no. 15 and 16).

4. TLR3 and IFN-stimulated gene (ISG) 20 in MCs

Interferon (IFN)-stimulated gene 20 (ISG20) is a 3′ - to - 5′ exonuclease specific for ssRNA and is involved in host defense reactions against RNA viruses [24, 25]. IFNs are key cytokines that regulate antiviral reactions and ISGs are class of major effector molecules for IFNs. Apart from antiviral reaction, ISGs may be involved in the pathogeneisis of a lupus model in mice [26]. We addressed the effect of poly IC on the expression of ISG20 in cultured MCs [17]. Poly IC treatment of MCs induced the expression of ISG20 in concentration- and time-dependent manners. Also, treatment of cells with poly IC induced the expression of IFN-β mRNA, but this was not the case with IFN-α. Transfection of the cells with siRNA against TLR3 or IRF3 suppressed the poly IC-induced expression of ISG20 mRNA and protein, while non-silencing control siRNA had no effect. On the other hand, siRNA against RIG-I, MDA5 or p65 did not affect the ISG20 expression (Figure 5.) [17]. Although siRNA may induce the expression of ISG20 nonspecifically, non-silencing control siRNA did not induce the expression of ISG20 under the condition we examined. Moreover, RNA interference against NF-κB p65 failed to inhibit poly IC-induced ISG20 expression.

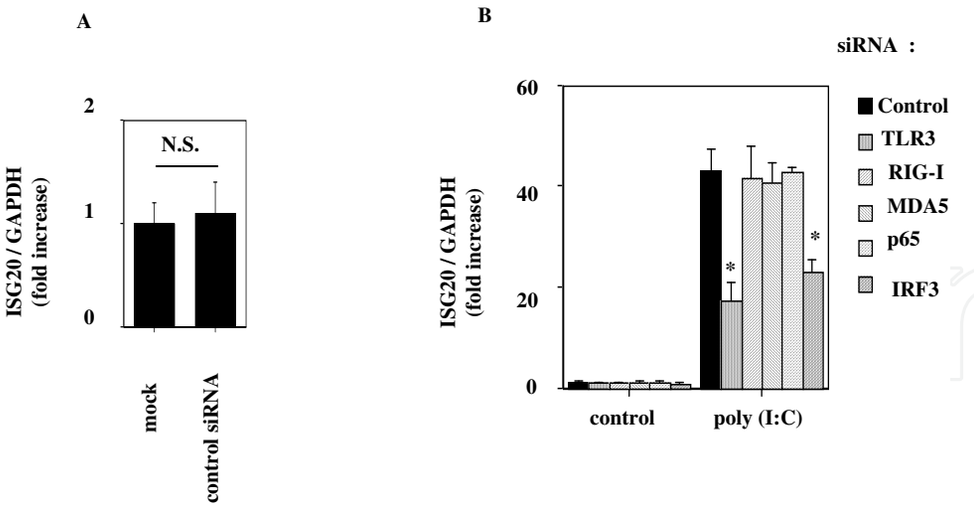

Figure 5. TLR3 and IRF3 are involved in poly IC-induced ISG20 expression. A. MCs were transfected with non-silencing control siRNA. After incubating for 24 h, RNA was extracted from cells. Expression of ISG20 mRNA was examined by real-time PCR. B. MCs were transfected with siRNA against TLR3, RIG-I, MDA5, p65, IRFr or a non-silencing control siRNA. 24 h after trasfections, the cells were treated for 16 h with 50 μg/ml poly IC and analyzed by real-time PCR for ISG20 mRNA (*p<0.01, n=3) (Imaizumi et al. Nephron Exp Nephrol 2011).

Transfection of the cells with IFN-β siRNA markedly inhibited the poly IC-induced expression of ISG20. Pretreatment of the cells with blocking antibody against type I IFN receptor also reduced the poly IC-induced expression of ISG20. Alternatively, transfection of the cells with an expression plasmid for IFN-β resulted in the over expression of ISG20 (Figure 6.) [17]. These observations suggest that *de novo* synthesized IFN-β is involved in poly IC-induced ISG20 expression.

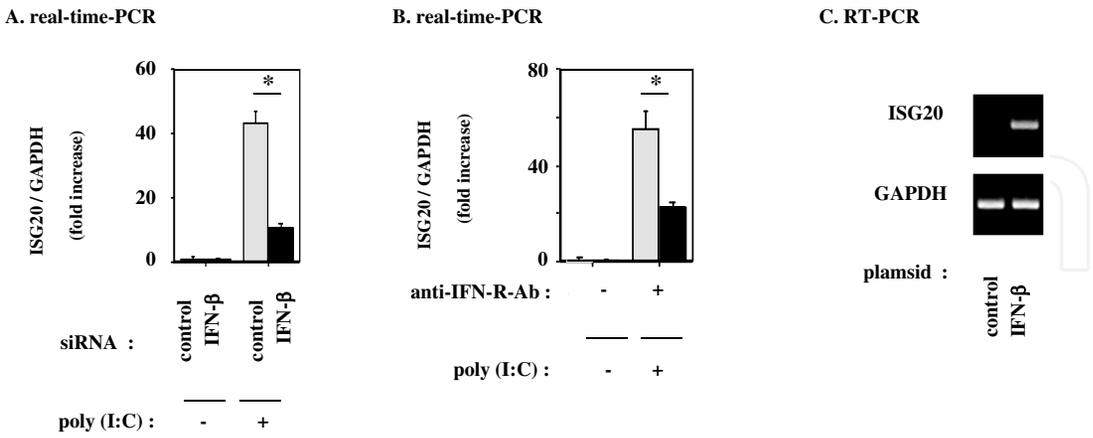

Figure 6. MCs were transfected with siRNA against IFN-β or a non-silencing control siRNA. A. After 24 h of transfection, the cells were treated with 50 μg/ml poly IC, RNA was extracted from cells after 16 h treatment with poly IC, and real-time PCR analysis for ISG20 was performed (*p<0.01, n=3). B. The cells were pretreated with a blocking antibody against type I IFN receptor (anti-IFN-ab) for 1 h, and subsequently treated with 50 μg/ml poly IC for 24 h (*p<0.01, n=3). C. The cells were transfected with an expression plasmid for IFN-β and incubated for 24 h (Imaizumi, et al. Nephron Exp Nephrol 2011).

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Dexamethasone inhibits the induction of IFN-β and ISG20 by poly IC, but it did not affect the expression of ISG20 by IFN-β. Thus, the inhibitory effect of dexamethasone may depend on the suppression of IFN-β production, which is consistent with our previous report on the proposed TLR3/IFN-β/RIG-I/CCL5 pathway [16]. Transfection of MCs with a poly IC/cationic lipid complex induced the expression of ISG20 mRNA and protein. Knockdown of RIG-I, but not of TLR3 or MDA5, inhibited the induction of ISG20 by poly IC/cationic lipid complex.

On the basis of these results, TLR3, IFR3 and *de novo* synthesized IFN-β may mediate the expression of ISG20 induced by the simply treatment of poly IC, while RIG-I, but not MDA5, may be involved in the expression of ISG20 induced by poly IC/cationic lipid complex in this setting using cultured normal human MCs [17]. A previous study showed the recognition of a poly IC/cationic lipid complex by MDA5, not by RIG-I, in murine MCs [6]. The molecular mechanisms of pathogen recognition may vary between species. Although clinical impact of ISG20 expression in MCs except for antiviral responses remains to be elucidated in future studies [26], we found the novel TLR3/IRF3/IFN-β/ISG20 pathway in poly IC signaling in MCs, and this pathway may play an important role in immune and inflammatory reactions against viral infection in MCs.

5. TLR3, MDA5 and RIG-I in MCs

Recently, it was shown that MDA5 and RIG-I function as pathogen recognition receptor against viral dsRNA in the cytosome, and both the receptor may play an important role in innate immune reactions [4, 5]. Although the expression of MDA has been documented in murine MCs [6], and human MCs [17], detailed implications for the expression of MDA5 in human MCs have not been clarified. Thus, we next examined the effect of poly IC and the role of MDA5 in C-X-C motif chemokine 10 (CXCL10) (or IFN-γ-induced protein 10, IP-10) expression in cultured human MCs [8]. Poly IC, either simply applied to the cells or transfected as a complex with a cationic lipid, induced MDA5 expression in concentration- and time-dependent manners. Transfection of the cells with siRNA against TLR3 suppressed the poly IC-induced expression of MDA5 mRNA and protein, while siRNA against TLR3 did not suppress the poly IC/cationic lipid complex-induced expression of MDA5. On the other hand, siRNA against RIG-I significantly inhibited the MDA5 expression induced by poly IC/cationic lipid complex (Figure 7.) Knockdown of MDA5 had no effects on the expression of RIG-I induced by poly IC or poly IC/cationic lipid complex (Figure 8.). Thus, MDA5 may be located in the downstream of RIG-I in this signaling pathway in cultured human MCs [8]. These results are inconsistent with a previous report dealing with MDA5 expression in murine MCs [6]. The molecular mechanisms of pathogen recognition may vary between species, although this issue remains to be elucidated in future studies [17].

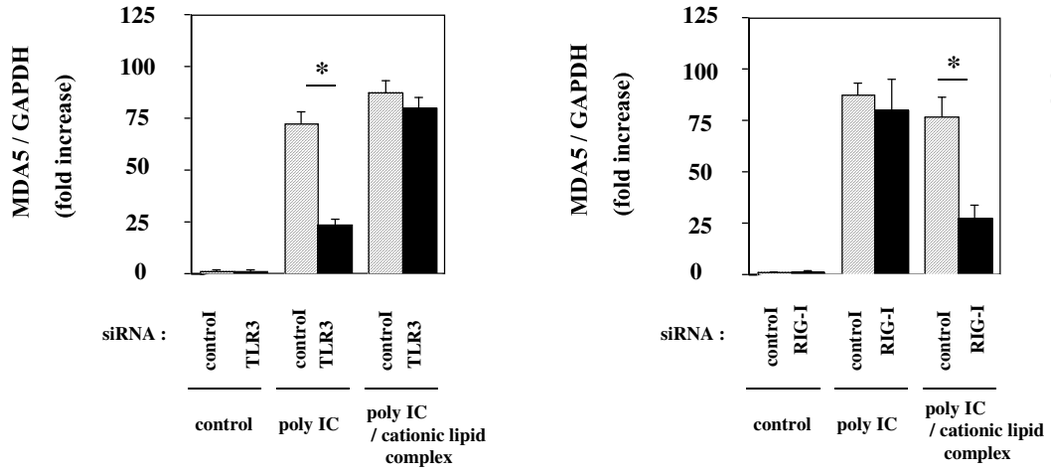

Figure 7. MCs were transfected with siRNA against TLR3, RIG-I, MDA5 or a non-silencing control siRNA. 24 h after transfection, the cells were treated with 30 μg/ml poly IC or were transfected with the complex of 1 ng/ml poly IC/ cationic lipid. After 16 h or 24 h incubation, the cells were subjected to real-time PCR analysis (Imaizumi et al. Tohoku J Exp Med 2012).

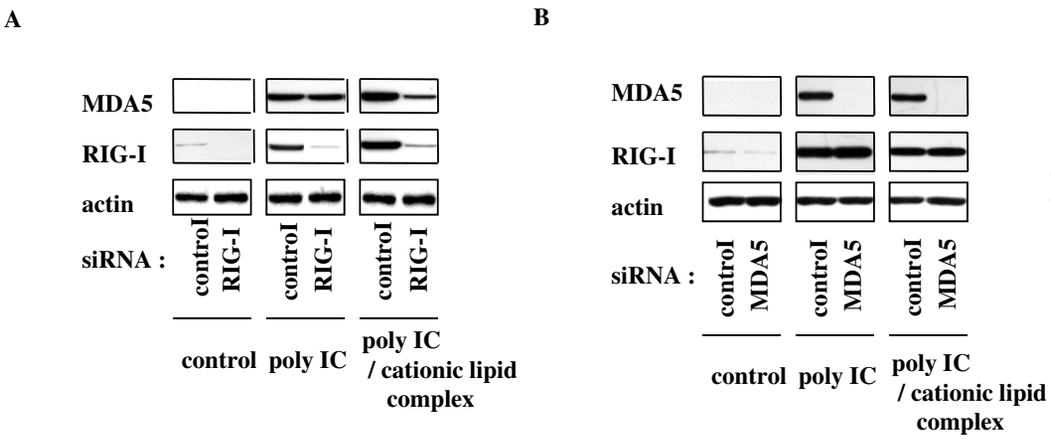

Figure 8. MCs were transfected with siRNA against RIG-I, MDA5, or non-silencing control siRNA. 24 h after transfection, the cells were treated with 30 μg/ml poly IC or were transfected with the complex of 1 ng/ml poly IC/cationic lipid. After 16 h or 24 h incubation, the cells were subjected to western blot analysis (Imaizumi et al. Tohoku J Exp Med 2012).

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Induction of IFN-β mRNA was observed in the cells treated with poly IC or those transfected with a poly IC/cationic lipid complex. In this experiment, TLR3 knockdown suppressed IFN-β induction in the poly IC-treated cells, while RIG-I knockdown suppressed the induction in the cells transfected with poly IC/cationic lipid. Transfection of the cells with IFN-β siRNA markedly inhibited production of MDA5 and CXCL10 induced by poly IC treatment or poly IC/cationic lipid transfection. On the other hand, MDA5 was markedly induced by the transfection with an IFN-β expression plasmid. Thus, it is considered that newly synthesized IFN-β mediates poly IC-induced MDA5 expression (Figure 9.). Apart from anti-viral property, IFN-β has been reported to be involved in the pathogenesis of autoimmune diseases. IFN-β is an important mediator in virus-associated glomerulonephritis and immune complex-mediated glomerulonephritis exacerbated by viral infections [27]. In our previous studies, poly IC treatment of MCs induced the expression of IFN-β and de novo synthesized IFN-β mediated the expressions of RIG-I and ISG20 [16, 17]. In the present study, we observed that IFN-β is induced either by poly IC or a poly IC/cationic lipid complex, and *de novo* synthesized IFN-β may mediate the expression of MDA5 [8]. RIG-I is involved in IFN-β expression induced by poly IC/cationic lipid complex, but not in the MDA expression by IFN-β. CXCL10, a chemokine with chemotactic activity for the leukocytes with CXCR3, is involved in the pathogenesis of glomerular diseases. MDA5 is known to mediate CXCL10 induction in human bronchial epithelial cells infected with Rhinovirus [28]. We observed that MDA5 is involved in the poly IC-mediated expression of CXCL10 in MCs. Further, we found that the TLR3/IFN-β/MDA5/CXCL10 pathway activates by poly IC treatment, while RIG-I/IFN-β/MDA5/CXCL10 pathway activates by poly IC/cationic lipid complex treatment in anti-viral and inflammatory reactions in MCs.

Stored kidney specimens in good condition obtained from 6 cases (diffuse proliferative lupus nephritis, 2; proteinuric IgA nephropathy, 2; minimal change nephrotic syndrome, 1; nutcracker syndrome, 1) were used for immunefluorescent study of MDA5 and RIG-I expression. After blocking by incubation with 1% goat serum, the slides were incubated with an anti-MDA5 antibody (1:100) or an anti-RIG-I antibody (1:1000). Intense MDA5 immunoreactivity was detected in MCs of the specimens from diffuse proliferative lupus nephritis and proteinuric IgA nephropathy, while the expression in non-immune complex mediated renal diseases was undetectable. Interestingly, RIG-I immunoreactivity was only in diffuse proriferative lupus nephritis (Figure 10.) [8].

In human subjects, mesangial expressions of TLR3 and RIG-I have been reported in patients with lupus nephritis [13, 18]. In addition, we observed mesangial MDA5 immunoreactivity in biopsy specimens from patients with severe lupus nephritis and proteinuric IgA nephropathy while no MDA5 expression in patients with non-inflammatory renal diseases [8]. Interestingly, there was no mesangial expression of RIG-I in the specimens from patients with IgA nephropathy, despite of positive staining of MDA5. These observations suggest the expression of MDA5 in severe lupus nephritis is associated with the activation of signaling pathway via RIG-I, but MDA expression in IgA nephropathy is independent on RIG-I, although this theory remains speculative. Differential roles of MDA5 and RIG-I in severe lupus nephritis and protenuric IgA nephropathy may predict specific molecular mechanisms for these glomerulonephritis. This should be further investigated in future studies.

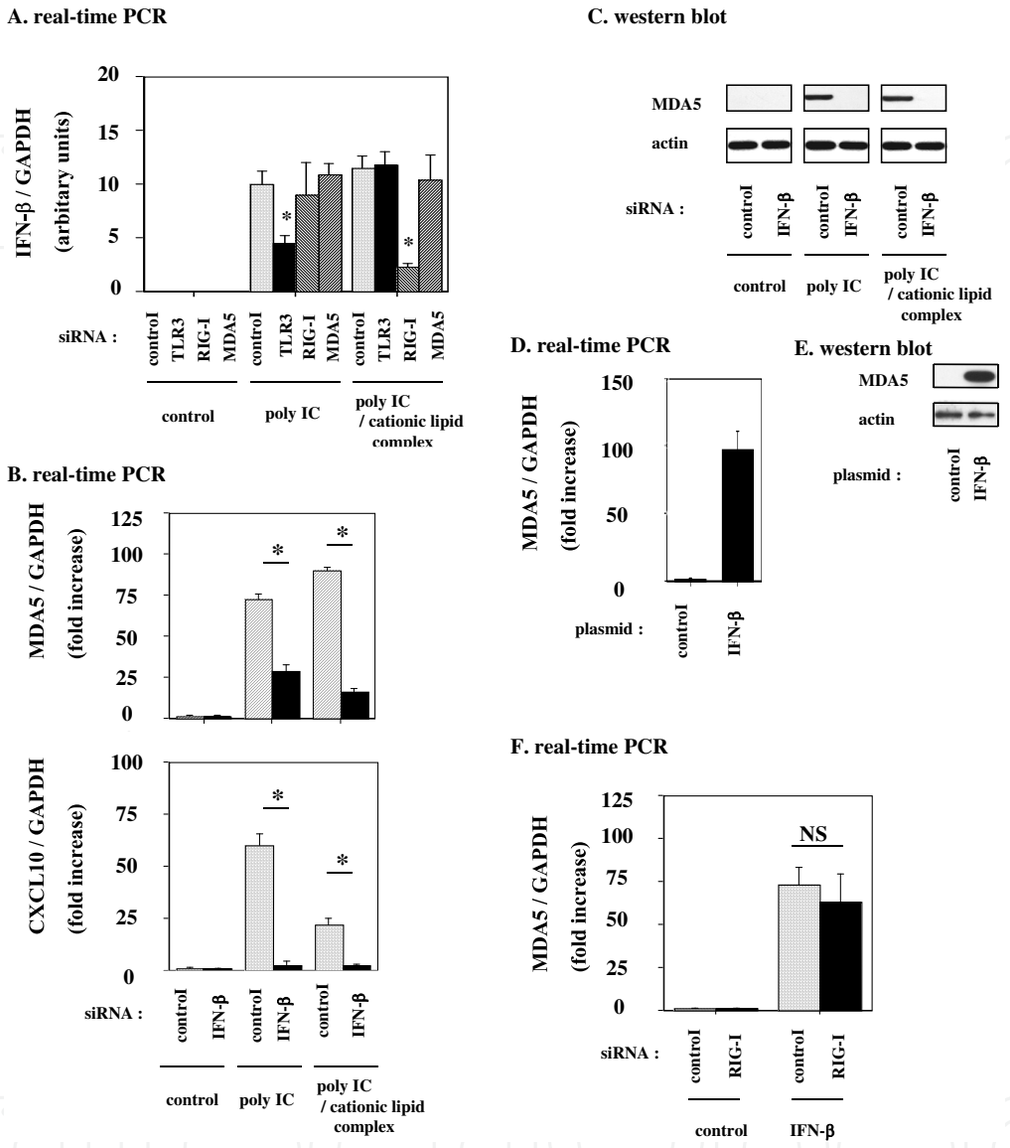

Figure 9. A. The cells were transfected with a non-silencing control siRNA, siRNA against TLR3, RIG-I or MDA5. After 24 h of transfection, the cells were treated or transfected with poly IC. RNA was extracted from cells after additional 4 h incubation, and the expression of IFN-β mRNA was examined using real-time PCR analysis (*p<0.01 vs. control, n=3). B and C. The cells were transfected with siRNA against IFN-β, and subsequently treated or transfected with poly IC. RNA was extracted after additional 16 h incubation, and real-time PCR analysis for MDA5 or CXCL10 was performed (*p<0.01, n=3). The cells were lysed after additional 24 h incubation, and lysates were subjected to western blot analysis for MDA5. The conditioned medium was collected, and the concentration of IFN-β was measured using an ELISA (*p<0.01, n=3). D and E. The cells were trenafected wit an expression plasmid for IFN-β and incubated for 24 h. Real-time PCR and western blot analysis for MDA5 were performed. F. The cells were transfected with siRNA aginst RIG-I.After 24 h incubation, the cells were treated with 10 ng/ml r(h) IFN-β for 8h. RNA was extracted from the cells and real-time PCR analysis for MDA5 was performed (Imaizumi et al. Tohoku J Exp Med 2012).

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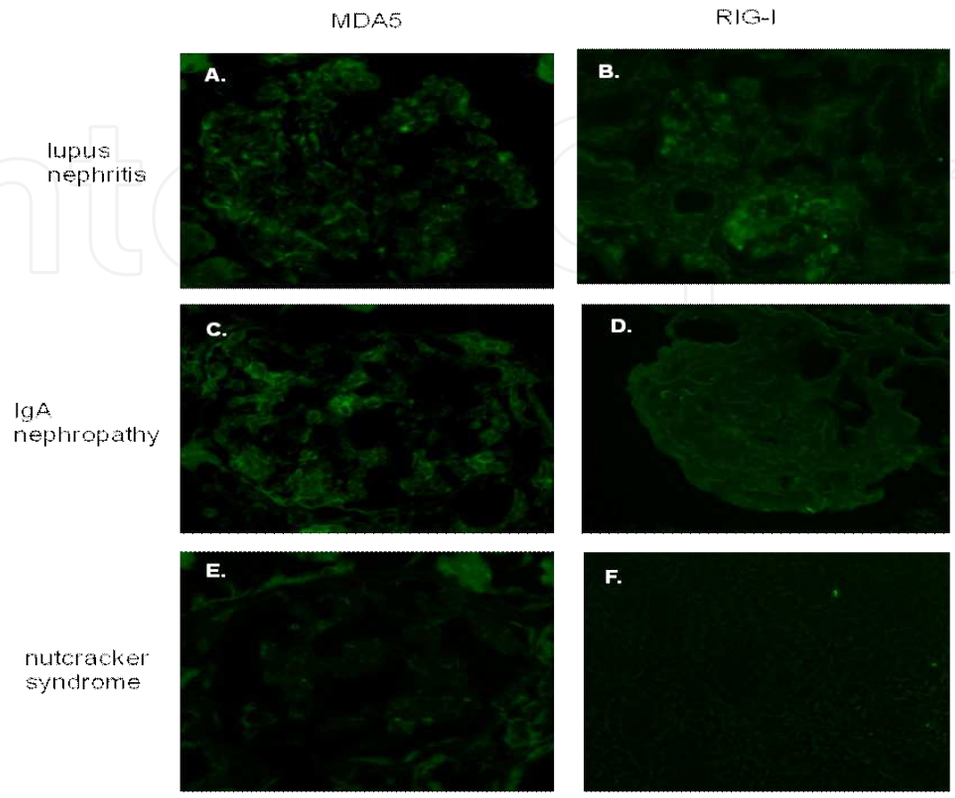

Figure 10. Intense MDA immunereactivity was detected in mesangial area of diffuse proliferative lupus nephritis (A) and proteinuric IgA nephropathty (C), but not in nutcracker syndrome (E). On the other hand, significant increase in the immunestaining intensity for RIG-I was observed only in severe lupus nephritis (B) (Imaizumi et al. Tohoku J Exp Med 2012).

6. Conclusion

We believe that involvement of the novel signaling pathways in MCs: the RIG-I-mediated IFN-γ/RIG-I/IRF7, TLR3/IFN-β/RIG-I/CCL5, RIG-I/IFN-β/MDA5/CXCL10 pathways, and the RIG-I-independent TLR3/IRF3/IFN-β/ISG20, and TLR3/IFN-β/MDA5/CXCL10 pathways may contribute to mesangial inflammation. Cross-talk of these signaling pathways may be involved in pathogenesis of human glomerulonephritis, and in the aggravation of glomerulonephritis due to viral infections. Although our findings remain preliminary, the intervention of these signaling pathways may lead to the development of future therapeutic strategies in the glomerular diseases. We found the involvement of novel RIG-I-mediated signaling pathways in mesangial inflammation in human MCs and there differences from TLR3 triggering, which addressed clinical significance.

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