Kidney & Blood Pressure Research

Kidney Blood Press Res 2019;44:62–71
DOI: 10.1159/000498837
Published online: 22 February 2019
Accepted: 5 February 2019

Research Article

Cytosolic Sensors of Viral RNA Are Involved in the Production of Interleukin-6 via Toll-Like Receptor 3 Signaling in Human Glomerular Endothelial Cells

Qiang Liu[a,b] Tadaatsu Imaizumi[c] Tomomi Aizawa[d] Koji Hirono[d] Shogo Kawaguchi[e] Shojiro Watanabe[d] Koji Tsugawa[d] Tomoh Matsumiya[c] Kazuhiko Seya[c] Hidemi Yoshida[c] Hiroshi Tanaka[d,f]

[a]Department of Pathology and Bioscience, Hirosaki University Graduate School of Medicine, Hirosaki, Japan, [b]Department of Nephrology, the First Hospital of China Medical University, Shenyang, China, [c]Department of Vascular Biology, Hirosaki University Graduate School of Medicine, Hirosaki, [d]Department of Pediatrics, Hirosaki University Hospital, Hirosaki, [e]Department of Gastroenterology, Hirosaki University Graduate School of Medicine, Hirosaki, [f]Department of School Health Science, Hirosaki University Faculty of Education, Hirosaki, Japan

Key Words
Glomerular endothelial cells • Interleukin-6 • Lupus nephritis • Melanoma differentiation associated gene 5 • Retinoic acid-inducible gene-I • Toll-like receptor 3

Abstract
Background/Aims: Dysregulation of interleukin-6 (IL-6) production in residual renal cells may play a pivotal role in the development of glomerulonephritis (GN). Given that Toll-like receptor 3 (TLR3) signaling has been implicated in the pathogenesis of some forms of GN, we examined activated TLR3-mediated IL-6 signaling in cultured normal human glomerular endothelial cells (GECs). ***Methods:*** We treated GECs with polyinosinic-polycytidylic acid (poly IC), an authentic double-stranded RNA, and analyzed the expression of IL-6 and the cytosolic viral RNA sensors retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation associated gene 5 (MDA5) using reverse transcription quantitative real-time polymerase chain reaction, western blotting, and enzyme-linked immunosorbent assays. To further elucidate the effects of poly IC on this signaling pathway, we subjected the cells to small interfering RNA (siRNA) against TLR3, interferon (IFN)-β, RIG-I, and MDA5. ***Results:*** We found that poly IC induced the expression of RIG-I, MDA5 and IL-6 via TLR3/IFN-β signaling in GECs. siRNA experiments revealed that both MDA5 and RIG-I were involved in the poly IC-induced expression of IL-6, with MDA5 being upstream of RIG-I. ***Conclusion***: Interestingly, cytosolic sensors of viral RNA were found to be involved in IL-6 production via TLR3 signaling in GECs. Regional activation of TLR3/IFN-β/

Hiroshi Tanaka, MD, PhD
Department of School Health Science, Hirosaki University Faculty of Education
Hirosaki 036-8560 (Japan)
E-Mail hirotana@hirosaki-u.ac.jp

MDA5/RIG-I/IL-6 axis due to viral and "pseudoviral" infections is involved in innate immunity and inflammatory reactions in GECs. We believe this signaling pathway also plays a pivotal role in the development of some forms of GN.

Introduction

In addition to contributing to the formation of the blood vessels in glomeruli, which act as a glomerular filtration barrier, glomerular endothelial cells (GECs) have been shown to produce various functional molecules that regulate aspects of glomerular function, including blood coagulation, inflammation, and homeostasis [1, 2]. Dysregulated endosomal Toll-like receptor (TLR) signaling has been reported to result in sustained activation of the type I interferon (IFN) system, which may lead to the development of autoimmune diseases such as systemic lupus erythematosus [3]. Thus, viral infections and antiviral host defenses may trigger the development of some inflammatory renal diseases, such as IgA nephropathy and lupus nephritis (LN) [4-6]. As GECs are directly exposed to circulating viral particles in the glomerulus [7], TLR3 and its signaling pathways involved in inflammatory processes in such cells are thought to play a pivotal role in the pathogenesis of some immune complex-mediated GN [7, 8] and viral infection-induced GN cases [9], However, little is known of the specific molecular mechanisms underlying the initiation of glomerular inflammation via endothelial TLR3 signaling activation [7, 8]. Since recruitment of circulating neutrophils by GECs is a first step in the development of GN [10], it is important to examine whether endothelial TLR3 signaling can promote circulating neutrophil recruitment. In previous work, we examined TLR3 signaling pathways in cultured human GECs, and found that activation of TLR3 induces endothelial expression of CXCL1, a potent neutrophil chemoattractant, and E-selectin, an adhesion molecule [8].

The representative proinflammatory cytokine interleukin-6 (IL-6) has been reported to be produced by residual glomerular cells in response to various inflammatory stimuli, and, notably, IL-6-dependent cellular crosstalk may modulate glomerular inflammation [1, 11, 12]. Interestingly, Kuravi et al. found that such cellular crosstalk between podocytes and GECs can regulate neutrophil recruitment by the latter [11]. However, the roles of endothelial TLR3 signaling and the cytosolic sensors retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) in controlling IL-6 expression remain to be elucidated. Given the importance of neutrophil recruitment in the pathogenesis of GN [10], we sought in the present study to determine the molecular mechanism by which TLR3 activation stimulates expression of IL-6 in human GECs.

Materials and Methods

Reagents

Polyinosinic-polycytidylic acid (poly IC, P1530) and a rabbit anti-actin antibody were purchased from Sigma (St. Louis, MO). Small-interfering RNAs (siRNAs) against TLR3 (SI02655156), MDA5 (SI03649037), and RIG-I (SI03019646) and a non-silencing negative-control siRNA (1027281) were obtained from Qiagen (Hilden, Germany). The IFN-β-targeting siRNA used has been described previously [8, 13-15]. An illustra RNA spin kit was supplied by GE Healthcare (Little Chalfont, UK). Custom oligonucleotide primers for reverse transcription (RT) and polymerase chain reaction (PCR) were synthesized by Fasmac (Atsugi, Japan). Moloney murine leukemia virus (MMLV) reverse transcriptase and Lipofectamine RNAi-MAX were provided from Invitrogen (Carlsbad, CA). Sso-Advanced Universal SYBR Green Supermix was obtained from Bio-Rad (Hercules, CA). A rabbit anti-MDA5 antibody (29020) was purchased from Immuno-Biological Laboratories (Takasaki, Japan), and the rabbit anti-RIG-I antibody used has been described in prior work [16]. An enzyme-linked immunosorbent assay (ELISA) kits for IL-6 and CXCL1 was supplied from Proteintech (Chicago, IL) and R&D systems (Minneapolis, MN), respectively

Cells

Normal human GECs were purchased from ScienCell (Carsbad, CA) and cultured in endothelial growth medium from Lonza (Walkersville, MD). The cells were treated with 0.1-50 μg/mL poly IC for up to 24 h [8]. In RNA interference experiments, the cells were transfected with non-silencing control siRNA or siRNA against TLR3, IFN-β, MDA5, or RIG-I using Lipofectamine RNAi-MAX regent according to the supplier's protocol. After incubation for 2 days, the cells were stimulated with 30 μg/mL poly IC.

Table 1. Oligonucleotide primers for reverse transcription quantitative real-time polymerase chain reaction

cDNA	primers
CXCL1	F: 5'- ATGGCCCGCGCTGCTCTCTCC -3
	R: 5'- GTTGGATTTGTCACTGTTCAG -3'
IFN-β	F: 5'- CCTGTGGCAATTGAATGGGAGGC -3'
	R: 5'- CCAGGCACAGTGACTGTACTCCTT -3'
IL-6	F: 5'- ATGAACTCCTTCTCCACAAGC -3'
	R: 5'- AAGAGCCCTCAGGCTGGACTG
GAPDH	F: 5'- GCACCGTCAAGGCTGAGAAC -3'
	R: 5'- ATGGTGGTGAAGACGCCAGT -3'
MDA5	F: 5'-GTTGAAAAGGCTGGCTGAAAAC -3'
	R: 5'-TCGATAACTCCTGAACCACTG-3'
RIG-I	F: 5'- GTGCAAAGCCTTGGCATGT -3'
	R: 5'- TGGCTTGGGATGTGGTCTACTC -3'
TLR3	F: 5'- CTCAGAAGATTACCAGCCGCC -3'
	R: 5'- CCATTATGAGACAGATCTAATG -3'

RNA extraction and reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR)

RNA was extracted from the cells and used to synthesize single-stranded cDNA using MMLV reverse transcriptase and oligo$(dT)_{18}$ primer. IFN-β, RIG-I, MDA5, or IL-6 cDNA was amplified using Sso-Advanced Universal SYBR Green Supermix. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as an internal control. The sequences of the primers used are shown in Table 1.

Western blotting

The cells were lysed using Laemmli reducing sample buffer, and the lysates subjected to polyacrylamide gel electrophoresis. The proteins were blotted onto polyvinylidene fluoride membranes, which were then blocked and probed with an antibody against RIG-I (1:10, 000), MDA5 (1:2, 000), or actin (1:3, 000). After washing, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit IgG antibody. Chemiluminescent substrate was used for detection.

Enzyme-linked immunosorbent assay (ELISA)

Concentrations of IL-6 and CXCL1 in the cell-conditioned medium were measured using commercial ELISA kits according to the supplier's recommended protocol.

Statistical analysis

All experiments were performed at least three times. Values are reported as means ± standard deviation (SD). Differences between groups were analyzed using Student's t-test. A p-value less than 0.05 was considered statistically significant. All analyses were carried out using GraphPad Prism software version 7 (GraphPad Software, Inc., La Jolla, CA).

Results

Poly IC induces the expression of RIG-I, MDA5 and IL-6 in cultured GECs

Treatment of cultured human GECs with poly IC induced the expression of RIG-I, MDA5, and IL-6 in a concentration- (Fig. 1) and time-dependent manner (Fig. 2). After stimulation with poly IC, expression of IFN-β mRNA rapidly increased, reaching its highest level after 2 h, then quickly decreasing and returning to the basal level at 8 h. Increased mRNA expression of RIG-I and MDA5 lagged behind that of IFN-β. Levels of RIG-I and MDA5 mRNA began to significantly increase 4 h after treatment, peaked at 16 h, and decreased thereafter. The level of IL-6 mRNA also reached its maximum at 16 h. RIG-I and MDA5 protein expression increased gradually and was highest at 16-24 h (Fig. 2B). IL-6 protein accumulated in the medium up to 24 h (Fig. 2C).

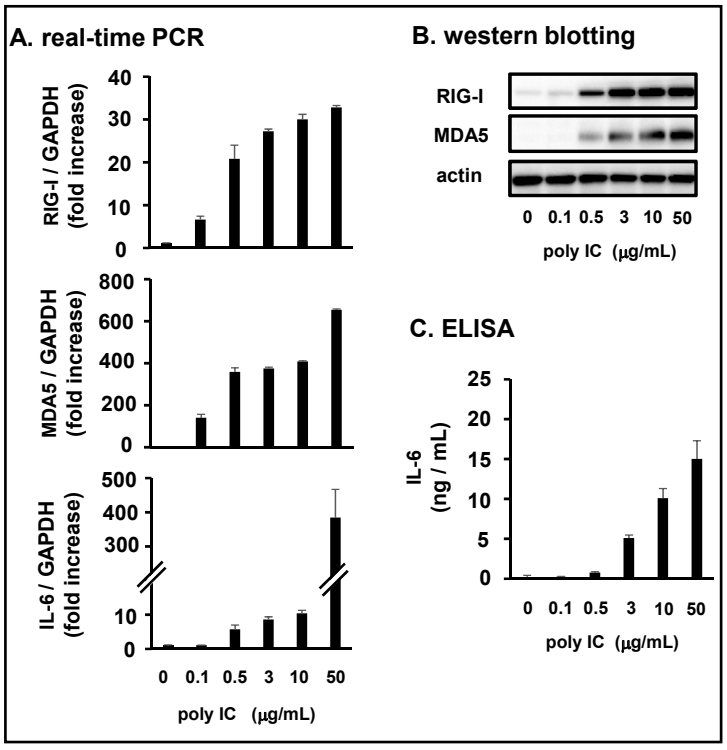

Fig. 1. Polyinosinic-polycytidylic acid (poly IC) induces the expression of retinoic acid-inducible gene-I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), and interleukin-6 (IL-6) in cultured human glomerular endothelial cells (GECs) in a concentration-dependent manner. GECs were cultured and treated with 0.1-50 μg/mL poly IC. (A) After 16 h of incubation, RNA was extracted from the cells. cDNA was synthesized and the expression of RIG-I, MDA5, or IL-6 mRNA was estimated using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) analysis. (B, C) After 24 h of incubation, the conditioned medium was collected and the cells were lysed. The lysates were subjected to western blotting for RIG-I, MDA5, and actin protein. The concentration of IL-6 in the collected medium was measured using an enzyme-linked immunosorbent assay (ELISA) kit. The data in (A) and (C) are means ± standard deviation (SD) (n=3).

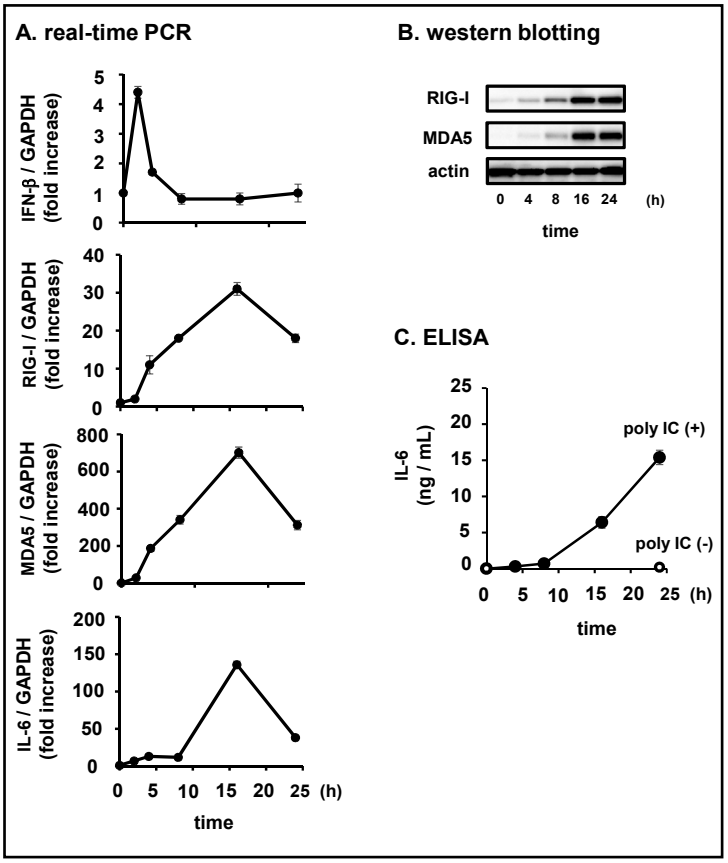

Fig. 2. Poly IC induces interferon (IFN)-β, RIG-I, MDA5, and IL-6 expression in a time dependent manner. The cells were treated with 30 μg /mL poly IC for up to 24 h. The mRNA (A) and/or protein (B, C) expression of IFN-β, RIG-I, MDA5, and IL-6 was examined as in Fig. 1. The data in (A) and (C) are means ± SD (n=3).

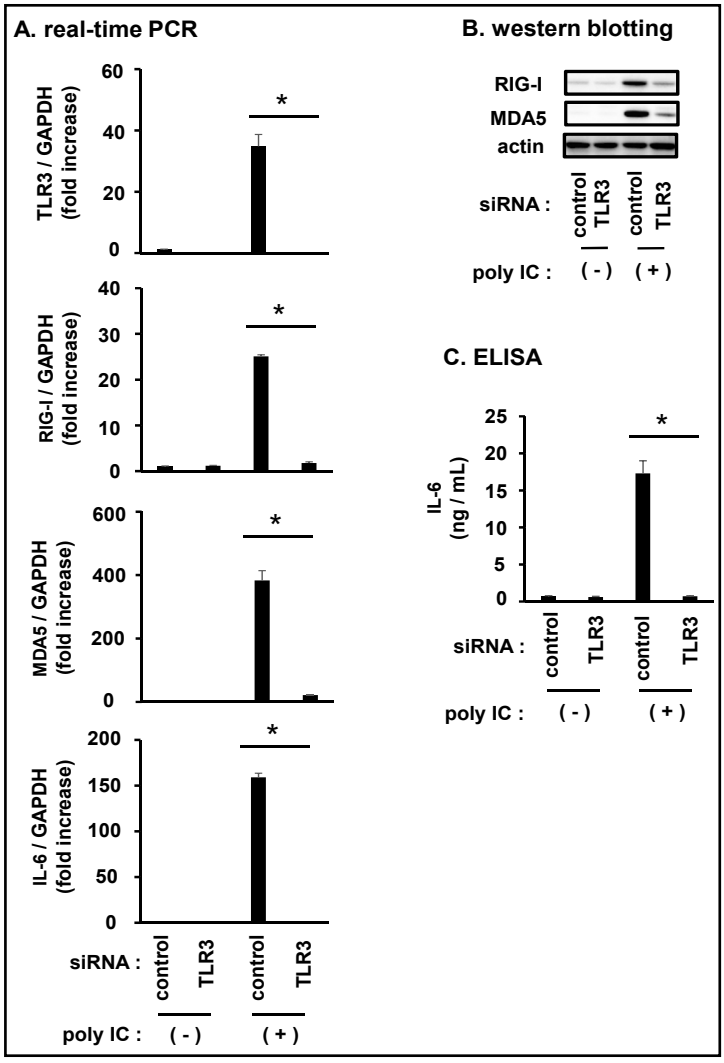

Fig. 3. Knockdown of Toll-like receptor 3 (TLR3) decreases the expression of RIG-I, MDA5, and IL-6 induced by poly IC. The cells were transfected with small interfering RNA against TLR3 and incubated for 48 h, before being treated with 30 μg/mL poly IC. (A) After an additional 16 h of incubation, RNA was extracted from the cells and RT-qPCR analysis was performed. (B and C) After an additional 24 h of incubation, the medium was collected and the cells were lysed. Western blotting analysis and ELISA were performed as in Fig. 1. The data in (A) and (C) are means ± SD (n=3). * p<0.01, t-test.

TLR3 and IFN-β mediate poly IC-induced expression of RIG-I, MDA5 and IL-6

RNA interference targeting TLR3 resulted in decrease in the RIG-I, MDA5, and IL-6 mRNA (Fig. 3A) and protein (Fig. 3B and 3C) expression induced by poly IC. Knockdown of IFN-β also inhibited the poly IC-induced expression of these molecules (Fig. 4).

MDA5 is involved in poly IC-induced RIG-I, IL-6 and CXCL1 expression

Knockdown of MDA5 diminished the expression of RIG-I, IL-6, and CXCL1 mRNA and protein induced by poly IC (Fig. 5).

RIG-I contributes to poly IC-induced expression of IL-6, but not that of MDA5 or CXCL1

RNA interference targeting RIG-I decreased the induction of IL-6 mRNA (Fig. 6A) and protein (Fig. 6C) expression in cells treated with poly IC. However, knockdown of RIG-I had no effect on poly IC induced increases in MDA5 and CXCL1 levels (Fig. 6A and 6B).

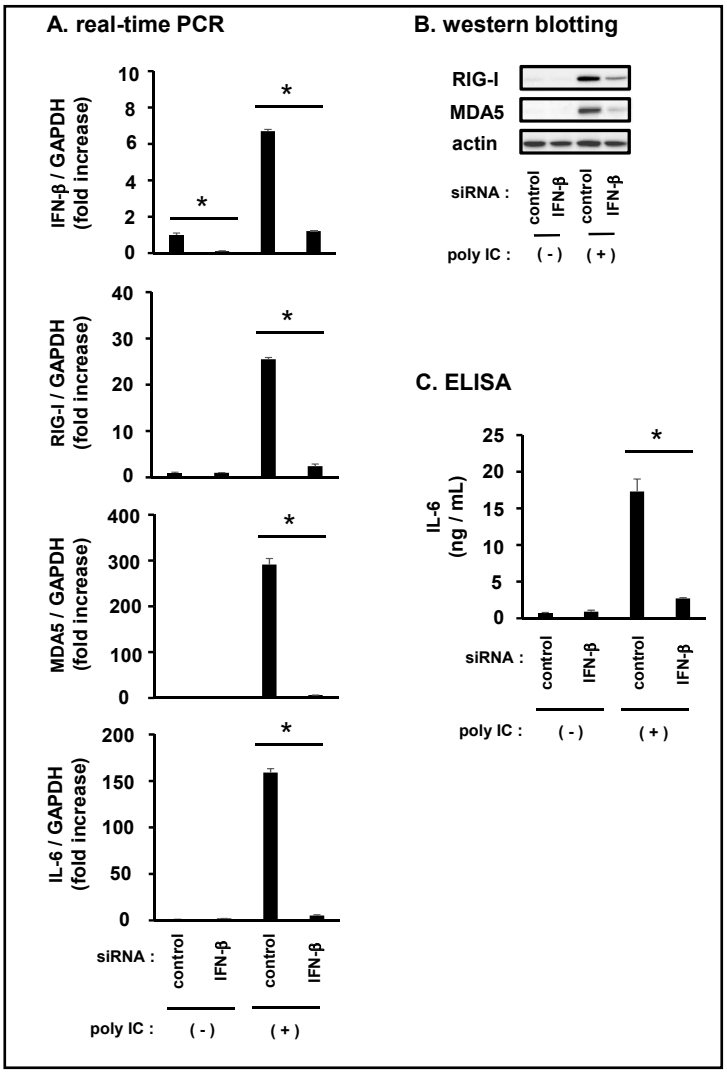

Fig. 4. IFN-β is involved in poly IC-induced expression of RIG-I, MDA5 and IL-6 RNA interference against IFN-β was performed as in Fig. 3, and RT-qPCR (A) western blotting (B) and ELISA (C) were performed as in Fig. 1. The data in (A) and (C) are means ± SD (n=3)* p<0.01, t-test.

Discussion

It has been reported that interaction between IL-6 and GECs modulates neutrophil recruitment and subsequent proinflammatory chemokine/cytokine secretion [12]. Since the recruitment of circulating neutrophils by GECs is a crucial first step in the development of GN [8, 11, 17], identifying the specific mechanisms in GECs responsible for this process is desirable for the development of therapeutic strategies for this disease [8]. In real-world clinical situations, viral infections occasionally trigger the development of GN or worsen pre-existing GN [4]. Notably, it has recently been reported that renal biopsy specimens from patients with LN exhibit increased expression of TLR3 compared to controls [18]. Furthermore, we previously noted intense glomerular expressions of RIG-I and MDA5, cytosolic sensors of viral RNA, in biopsy specimens from patients with proliferative LN, but only MDA5 and not RIG-I expression in specimens from patients with IgA nephropathy [19, 20]. Thus, activation of innate immunity, including the TLR3/RIG-I/MDA5 signaling axis, in the inflammatory process in residual glomerular cells is likely to be involved in the pathogenesis of some forms of GN, especially LN [5-8, 15]. With respect to the recruitment of circulating neutrophils by GECs, we previously observed that activation of TLR3/IFN-β signaling induces endothelial expression of CXCL1 and E-selectin [8], although this finding remains preliminary. However, the specific mechanisms underlying the induction of IL-6

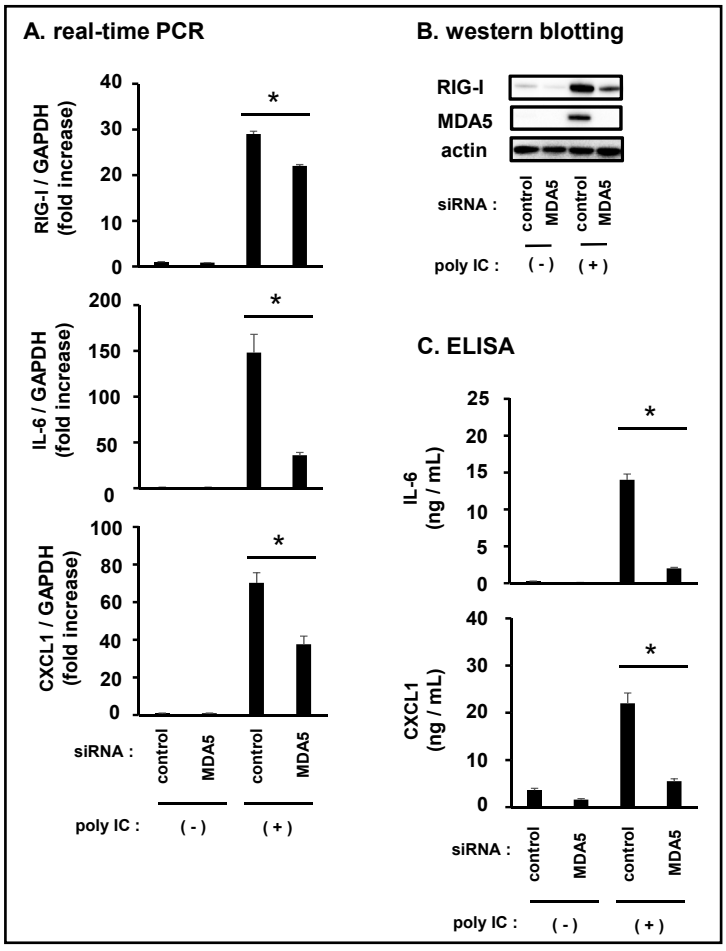

Fig. 5. MDA5 mediates the expression of RIG-I, IL-6, and CXCL1 induced by poly IC. RNA interference against MDA5 in cells, RT-qPCR (A), western blotting (B), and ELISA (C) were performed as above. Knockdown of MDA5 resulted in decreases in the poly IC-induced expression of RIG-I, IL-6, and CXCL1. The data in (A) and (C) are means ± SD (n=3). * $p<0.01$, t-test.

production via TLR3 signaling in GECs remain unknown. Thus, in the present study, we aimed to examine this issue.

Here, we found that poly IC treatment induced the expression of IL-6 in GECs in a time- and concentration-dependent manner. Experiments using siRNA confirmed that this response was mediated by signaling via TLR3. Concerning type I IFNs, we have shown in prior works using cultured human GECs and mesangial cells that IFN-β synthesized *de novo* following TLR3 activation is a key in regional inflammatory cascades [8, 13-15, 20]. Indeed, induction of RIG-I, MDA5, and IL-6 expression was lagged behind the increase in IFN-β levels observed here, and knockdown of IFN-β resulted in decreased expression of these molecules. These results suggest that IFN-β also mediates the increased expression of RIG-I, MDA5, and IL-6 in GECs treated with poly IC. Thus, various inflammatory molecules downstream of endothelial TLR3/IFN-β signaling may be involved in innate immune and inflammatory reactions and play a pivotal role, at least in part, in the subsequent recruitment of circulating neutrophils by GECs [8].

IL-6 is a pleiotropic cytokine that regulates numerous biological processes including inflammatory responses. Serum IL-6 level has been shown to correlate with the histologic severity of LN in a clinical context [21]. Of the residual glomerular cells, IL-6 can be produced by podocytes, mesangial cells, and GECs, and is involved in the pathogenesis of inflammatory renal diseases, including IgA nephropathy, LN, acute kidney injury, and chronic renal disease [12, 22]. With respect to the implication of cytosolic sensors of viral RNA within target cells in the secretion of IL-6, it has been reported that dengue virus induces IL-6 expression in dendritic cells via RIG-I and MDA5 [23]. In the present study, we also found that both

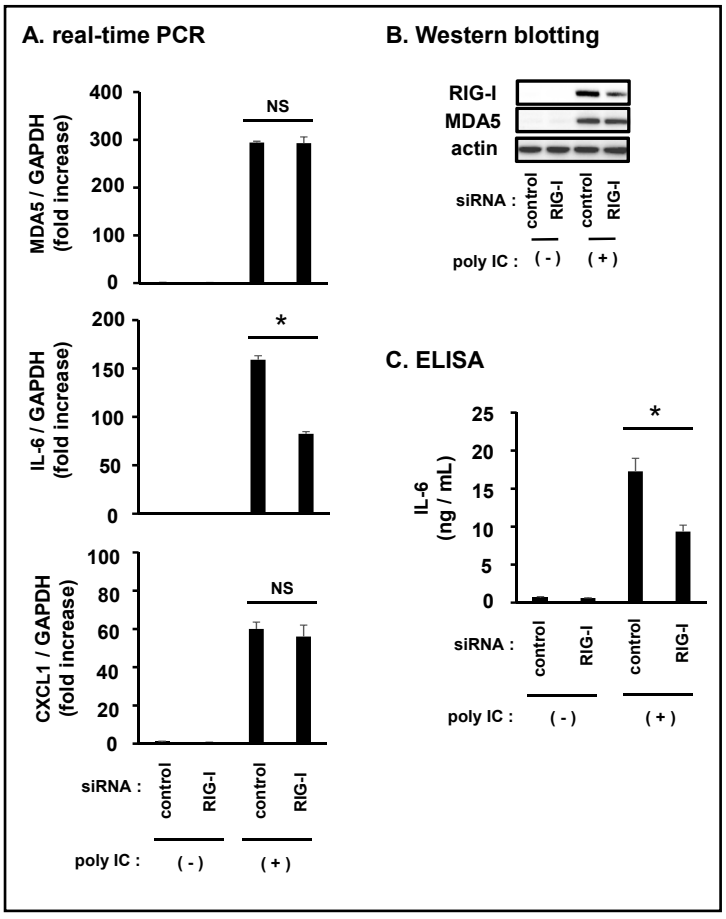

Fig. 6. RIG-I is involved in poly IC-induced expression of IL-6, but not that of CXCL1. RNA interference against RIG-I was performed as in Fig. 3. RT-qPCR (A), western blotting (B), and ELISA (C) were performed as above. Knockdown of RIG-I decreased poly IC-induced mRNA expression of IL-6, but not that of CXCL1 (A). The data in (A) and (C) are means ± SD (n=3). * p<0.01, t-test. NS; not significant.

RIG-I and MDA5 were involved in IL-6 production in GECs treated with poly IC. However, Hägele et al. previously reported that RIG-I, but not MDA5, contributes to poly IC/cationic lipid complex-induced IL-6 expression in murine GECs [7]. Although the reason for this discrepancy is not known, human and murine GECs may have different characteristics. In addition, the signaling induced by pure poly IC may differ from that induced by poly IC/cationic lipid complex, although this requires further examination.

Interestingly, knockdown of MDA5 decreased the poly IC-induced expression of RIG-I and CXCL1 in addition to that of IL-6, while knockdown of RIG-I affected the expression of neither MDA5 nor CXCL1. This suggests that MDA5 is, at least partially, upstream of RIG-I/IL-6, and the expression of IL-6 and CXCL1 are differentially regulated under the conditions of our experiment. The TLR3/IFN-β/MDA5/RIG-I/IL-6 axis may play a

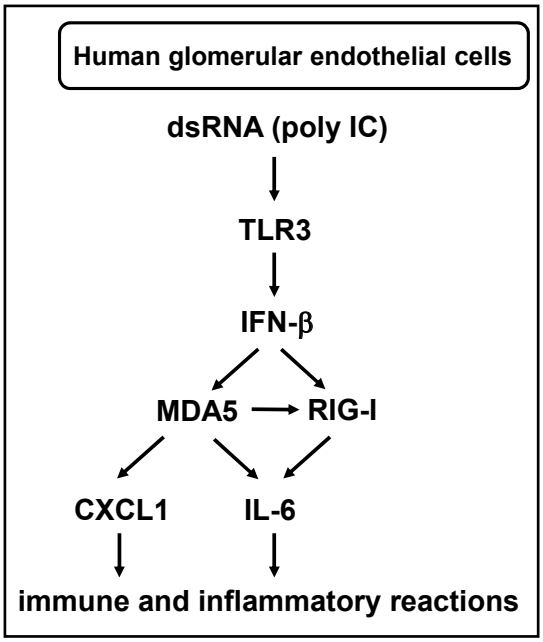

Fig.7. Proposed signaling axis in TLR3-mediated IL-6/CXCL1 expression in human glomerular endothelial cells.

role in inflammatory reactions related to viral and 'pseudoviral" infection in GECs, and IL-6 produced by such cells may induce immune and inflammatory reactions in other resident glomerular cells [11, 12]. Since GECs have been reported to interact with other intrinsic residual glomerular cells [1, 2, 11], initial regional neutrophil recruitment by GECs followed by TLR3 activation could trigger the development of GN, as is thought to occur in LN [5, 6, 8, 22]. Thus, identifying the specific mechanisms that underlie endothelial TLR3 signaling for the recruitment of circulating neutrophils via CXCL1, E-selectin, and IL-6 expression is desirable for the development of therapeutic strategies for GN [8, 22]. On the other hand, it is notably that TLR3 signaling has both protective and deleterious effects in the pathogenesis of virus and "pseudoviral" infections [24]. Also, TLR3 agonist poly IC used in this experiment sometimes promote apoptosis and increase cellular permeability in some cell types [24]. Thus, we should be careful to interpret our preliminary findings.

Conclusion

In conclusion, we found that the TLR3/IFN-β/MDA5/RIG-I/IL-6 axis probably contributes to postulated regional neutrophil recruitment and inflammation by GECs (Fig. 7). However, further studies, including those involving *in vivo* murine models, are required to confirm and extend our preliminary findings.

Acknowledgements

This study was supported by Grants-in-Aid from the Japan Society for Promotion of Science (JSPS KAKENHI Grant Number: 16K10055 to H.T. and 17K09681 to T.I.). This study was not involved human subjects which required ethical approval.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

1 Fogo AB, Kon A: The glomerulus - a view from the inside – the endothelial cell. Int J Biochem Cell Biol 2010;42:1388-1397.
2 Kitching AR, Hutton HL: The players: Cells involved in glomerular disease. Clin J Am Soc Nephrol 2016;11:1664-1674.
3 Hagberg N, Rönnblom L: Systemic lupus erythematosus - A disease with a dysregulated type I interferon system. Scand J Immunol 2015;82:199-207.
4 Lai AS, Lai KN: Viral nephropathy. Nat Clin Pract Nephrol 2006;2:254–262.
5 Anders HS: Pseudoviral immunity- a novel concept for lupus. Trend Mol Med 2009;15:553–561.
6 Tanaka H, Imaizumi T: Inflammatory chemokine expression via Toll-like receptor 3 signaling in normal human mesangial cells. Clin Dev Immunol 2013;2013:984708.
7 Hägele H, Allam R, Pawar RD, Anders HJ: Double-stranded RNA activates type I interferon secretion in glomerular endothelial cells via retinoic acid-inducible gene (RIG)-I. Nephrol Dial Transplant 2009;24:3312-3318,
8 Liu Q, Imaizumi T, Kawaguchi S, Aizawa T, Matsumiya T, Watanabe S, Tsugawa K, Yoshida H, Tsuruga K, Joh K, Kijima H, Tanaka H: Toll-like receptor 3 signaling contributes to regional neutrophil recruitment in cultured human glomerular endothelial cells. Nephron 2018;139:349-358.

9 Wörnle M, Schmid H, Banas B, Merkle M, Henger A, Roeder M, Blattner S, Bock E, Kertzler M, Gröne HJ, Schlöndrrff D: Novel role of Toll-like receptor 3 in hepatitis C-associated glomerulonephritis. Am J Pathol 2006;168:370-385.

10 Sadik CD, Kim ND, Luster AD: Neutrophil cascading their way to inflammation. Trend Immunol 2011;32:452-460.

11 Kuravi SJ, McGettrick HM, Satchell SC, Saleem MA, Harper L, Williams JM, Rainger GE, Savage COS: Podocytes regulate neutrophil recruitment by glomerular endothelial cells via IL-6–mediated crosstalk. J Immunol 2014;193:234-243.

12 Su H, Lei CT, Zhang C: Interleukin-6 signaling pathway and its role in kidney disease: An update. Front Immunol 2017 8:405.

13 Imaizumi T, Tanaka H, Matsumiya T, Yoshida H, Tanji K, Tsuruga K, Oki E, Aizawa-Yashiro T, Ito E, Satoh K: Retinoic acid-inducible gene-I is induced by double-stranded RNA and regulates the expression of CC chemokine ligand (CCL) 5 in human mesangial cells. Nephrol Dial Transplant 2010;25:3534-3539.

14 Imaizumi T, Yano C, Numata A, Tsugawa K, Hayakari R, Matsumiya T, Yoshida H, Watanabe S, Tsuruga K, Kawaguchi S, Murakami M, Tanaka H: Interferon (IFN)-induced protein 35 (IFI35), a type I interferon-dependent transcript, upregulates inflammatory signaling pathways by activating Toll-like receptor 3 in human mesangial cells. Kidney Blood Press Res 2016;41:635-642.

15 Imaizumi T, Hayakari R, Watanabe S, Aizawa T, Matsumiya T, Yoshida H, Tsuruga K, Kawaguch S, Tanaka H: Cylindromatosis (CYLD), a deubiquitinase, attenuates inflammatory signaling pathways by activating Toll-like receptor 3 in human mesangial cells. Kidney Blood Press Res 2017;42:942-950.

16 Imaizumi T, Aratani S, Nakajima T, Carlson M, Matsumiya T, Tanji K, Ookawa K, Yoshida H, Tsuchida S, McIntyre TM, Prescott SM, Zimmerman GA, Satoh K: Retinoic acid-inducible gene-I (RIG-I) is induced in endothelial cells by LPS and regulates expression of COX-2. Biochem Biophys Res Commun 2002;292:274-279.

17 Murakami S, Morioka T, Nakagawa Y, Suzuki Y, Arakawa M, Oite T: Expression of adhesion molecules by cultured glomerular endothelial cells in response to cytokines: Comparison to human umbilical vein and dermal microvascular endothelial cells. Microvasc Res 2001;62:383-391.

18 Conti F, Spinelli FR, Truglia S, Miranda F, Alessandri C, Ceccarelli F, Bombardieri M, Giannakakis K, Valesini G: Kidney expression of Toll-like receptors in lupus nephritis: Quantification and clinicopathological correlations. Mediators Inflamm 2016;2016:7697592.

19 Suzuki K, Imaizumi T, Oki E, Tsugawa K, Ito E, Tanaka H: Expression of retinoic acid-inducible gene-I in lupus nephritis. Nephrol Dial Transplant 2007;22:2407-2409.

20 Imaizumi T, Aizawa-Yashiro T, Tsuruga K, Tanaka H, Matsumiya T, Yoshida H, Tatsuta T, Xing F, Hayakari R, Satoh K: Melanoma differentiation-associated gene 5 regulates the expression of a chemokine CXCL10 in human mesangial cells: implications for chronic inflammatory renal diseases. Tohoku J Exp Med 2012;228:17-26.

21 Galil SMA, Ezzelden N, El-Boshy ME: The role of serum IL-17 and IL-6 as biomarkers of disease activity and predictors of remission in patients with lupus nephritis. Cytokine 2015;76:280-287.

22 Yung S, Cheung FK, Zhang Q, Chan TM: Mediators of inflammation and their effect on resident renal cells: Implications in lupus nephritis. Clin Dev Immunol 2013;2013:317682.

23 Sprokholt JK, Kaptein TM, van Hamme JL, Overmars RJ, Gringhuis SI, Geijtenbeek TBH: RIG-I-like receptor triggering by Dengue virus drives dendritic cell immune activation and T_H1 differentiation. J Immunol 2017;198:4764-477.

24 Patel MC, Shirey KA, Pletneva LM, Boukhvalova MS, Garzino-Demo A, Vogel SN, Blanco JCG: Novel drugs targeting Toll-like receptors for antiviral therapy. Future Virol 2014;9:811-829.