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INNATE IMMUNE REACTIONS AGAINST RNA VIRUSES IN RENAL MESANGIAL CELLS

Tadaatsu Imaizumi[1)], Tomomi Aizawa[2)], Hiroshi Tanaka[2,3)], Fuyuki Sato[4)], Fei Xing[1)], Tomoh Matsumiya[1)], HidemiYoshida[1)] and Kei Satoh[1)]

Abstract Viral infection is important in renal pathology both as a trigger of chronic inflammatory diseases and as a complication associated with organ transplantation. Glomerular mesangial cells produce a variety of functional molecules potentially involved in immune reactions, and we investigated anti-viral responses in normal human mesangial cells. Human mesangial cells were treated with polyinosinic-polycytidylic acid (poly IC), an authentic double-stranded RNA that mimics viral RNA. Treatment of cells with poly IC induced interferon-β (IFN-β), retinoic acid-inducible gene-I (RIG-I), CC chemokine ligand 5 (CCL5), differentiated embryo-chondrocyte 2 (DEC2) and IFN-stimulated gene 20 (ISG20). Knockdown of toll-like receptor 3 (TLR3), by RNA interference (RNAi), abolished the poly IC-induced expression of these molecules. RNAi against IFN-β inhibited the induction of RIG-I, CCL5 and ISG20, but not of DEC2. Knockdown of RIG-I resulted in the reduced expression of CCL5. RNAi against DEC2 enhanced the poly IC-induced expression of IFN-β, RIG-I and CCL5. Transfection of cells with a poly IC/cationic lipid complex induced IFN-β, RIG-I and ISG20. Knockdown of RIG-I decreased the expression of IFN-β and ISG20 induced by transfection with poly IC/cationic lipid. TLR3 and RIG-I may function as recognition receptors against double-stranded RNA, which induce IFN-β and its downstream IFN-inducible genes. In the signaling elicited by poly IC, the IFN-inducible genes include RIG-I and effector molecules as CCL5 with leukocyte chemotactic activity and ISG20 with exonuclease activity on single-stranded RNA. The poly IC-induced expression of DEC2 is independent on IFN-β and it may control the signaling elicited by double-stranded RNA. The poly IC-inducible molecules may mediate anti-viral innate responses in renal mesangial cells.

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Key words: TLR3; IFN-β; RIG-I; ISG20; DEC2; mesangial cells

Introduction

It is well known that viral infection can be a trigger of onset or worsening of chronic inflammatory renal diseases as lupus nephritis and IgA nephropathy. Viral infection is also an important complication associated with organ transplantation[1)]. Glomerular mesangial cells produce various functional molecules that regulate immune and inflammatory reactions. Thus the cells may also recognize invading viruses and play a part in the regulation of anti-viral immune responses in the kidney. Polyinosinic-polycytidylic acid (poly IC) is an authentic double-stranded RNA which induces anti-viral responses when applied to cells. Treatment of cells with poly IC may mimic the exposure of cells to double-stranded RNA released from virus-infected cells, and transfection of cells with a poly IC/cationic lipid complex is regarded as a model for cytoplasmic viral infection. Using these models, we have investigated anti-viral responses in normal human mesangial cells in culture.

[1)] Department of Vascular Biology, Hirosaki University Graduate School of Medicine
[2)] Department of Pediatrics, Hirosaki University Graduate School of Medicine
[3)] Department of School Health Science, Faculty of Education, Hirosaki University
[4)] Department of Pathology and Bioscience, Hirosaki University Graduate School of Medicine
Corresponding author: Tadaatsu Imaizumi, M.D.,
FAX: +81-172-39-5135
E-mail: timaizum@cc.hirosaki-u.ac.jp

Poly IC induces various genes in mesangial cells

Treatment of mesangial cells with poly IC induces interferon-β (IFN-β), retinoic acid-inducible gene-I (RIG-I; not "one" but "ai")[2], CC chemokine ligand 5 (CCL5)[2], differentiated embryo-chondrocyte 2 (DEC2)[3], and IFN-stimulated gene 20 (ISG20)[4]. Transfection of cells with a poly IC/cationic lipid complex also induces IFN-β, RIG-I and ISG20 [4], but not CCL5 or DEC2. Therefore, poly IC may activate multiple signaling cascades depending on the treatment or transfection of cells with poly IC, and the poly IC-inducible genes may be involved in anti-viral reactions in renal mesangial cells.

Role of toll-like receptor (TLR) 3 in poly IC-induced gene expression in mesangial cells

Innate immune reactions are initiated upon the recognition of pathogen-derived molecules by pattern recognition receptors, which lead to the activation of the subsequent downstream signaling cascades. TLRs are a family of the pattern recognition receptors. TLR3 is known to recognize double-stranded RNA of RNA viruses and then TLR3 activates the signaling to induce anti-viral responses [5]. RNA interference (RNAi) technique against TLR3 abolishes the expression of IFN-β, RIG-I, CCL5, DEC2 and ISG20 in the cells treated with poly IC [2-4]. TLR3 functions as a recognition receptor for poly IC; however, when the cells were transfected with poly IC/cationic lipid, TLR3 knockdown does not affect the expression of IFN-β and ISG20 [4].

RIG-I expression in mesangial cells treated or transfected with poly IC

RIG-I is a member of DExH box proteins and designated as a putative RNA helicase [6, 7]. RIG-I expression is enhanced in mesangial cells treated or transfected with poly IC [2, 4]. In our previous studies, poly IC was found to induce RIG-I expression in vascular endothelial cells [8], astrocytes [9] and gingival fibroblasts [10]; and RIG-I may be involved in anti-viral innate immune reactions in a wide variety of cell types.

RIG-I is known to serve as one of the cytoplasmic pathogen recognition receptors for double-stranded RNA of RNA viruses and thus initiates anti-viral responses including IFN-β production [11]. We demonstrated that RNAi against RIG-I decreases the expression of IFN-β and ISG20 in mesangial cells transfected with a poly IC/cationic lipid complex [4], and RIG-I may function as a cytoplasmic receptor for poly IC. In mesangial cells simply treated with poly IC, RIG-I knockdown inhibited the expression of CCL5 but not of IFN-β or ISG20; and in this model, RIG-I may serve as one of the poly IC signaling molecules but not as a recognition receptor. In our previous study, RNAi aganist RIG-I inhibited the poly IC-induced expression of CCL5 in U373MG astrocytoma cells [9], and CCL5 may function as a common effector molecule of poly IC-induced inflammatory responses in a wide range of tissues.

RIG-I is also induced in vascular endothelial cells treated with *E. coli* lipopolysaccharide [6] and in the liver and spleen of mice infected with *Listeria monocytogenes* [12], suggesting that RIG-I plays a role not only in anti-viral responses but in immune reactions against bacterial infection.

Histochemical studies revealed enhanced expression RIG-I in clinical samples as rheumatoid synoviocytes [13] and epidermis from psoriasis patients [14]. Enhanced expression of RIG-I protein in the glomerulus is demonstrated in biopsy specimens from lupus nephritis patients [15] and the levels of RIG-I mRNA is also enhanced in urinary sediment from such patients [16]. RIG-I may be involved in the pathogenesis of chronic inflammatory diseases of the kidney and

other organs.

Role of IFN-β in the expression of poly IC-inducible genes in mesangial cells

IFN-β is a key cytokine in anti-viral immune reactions and its biological effects are mediated by various ISGs. In mesangial cells, RNAi against IFN-β inhibited the poly IC-induced expression of RIG-I, CCL5 and ISG20, but not of DEC2; and newly synthesized IFN-β mediates, at least in part, the poly IC-induced expression of these genes. The poly IC-induced expression of IFN-β is decreased by pretreatment of cells with an anti-inflammatory steroid dexamethasone [4], and part of anti-inflammatory effects of dexamethasone depends on the inhibition of IFN-β production.

DEC2 is a basic-helix-loop-helix transcriptional factor [17], and poly IC treatment also induces DEC2 in mesangial cells [3]. RNAi against DEC2 enhances the poly IC-induced expression of IFN-β and its downstream genes, RIG-I and CCL5. Therefore, DEC2 may constitute a negative feedback system for the TLR3/IFN-β/RIG-I/CCL5 pathway, which may play a role in controlling protracted inflammatory reactions in mesangial cells.

ISG20 is induced by poly IC in mesangial cells

ISG20 is a 3'-to-5' exonuclease specific for single-stranded RNA and degrades viral RNA. ISG20 is induced both by poly IC treatment of mesangial cells and by transfection of the cells with a poly IC/cationic lipid complex. The induction of ISG20 is inhibited by knockdown of IFN-β, and the ISG20 may be involved in anti-viral reactions mediated through both the TLR3/IFN-β and RIG-I/IFN-β pathways in mesangial cells.

Conclusion

Poly IC is recognized by renal mesangial cells and enhances the expression of IFN-β, RIG-I, CCL5, DEC2 and ISG20. Induction of CCL5 and DEC2 was observed only in the cells treated with poly IC but not in those transfected with a poly IC/cationic lipid complex. We propose three signaling pathways, being consisting of TLR3/IFN-β/RIG-I/CCL5, TLR3/IFN-β/ISG20 or RIG-I/IFN-β/ISG20 (Figure 1), which may potentially mediate anti-viral responses in mesangial cells. DEC2 may also play a role in the anti-viral responses by negatively regulating the IFN-β/RIG-I/CCL5 pathway. These poly IC-inducible molecules may cooperate and regulate the anti-viral reactions in mesangial cells.

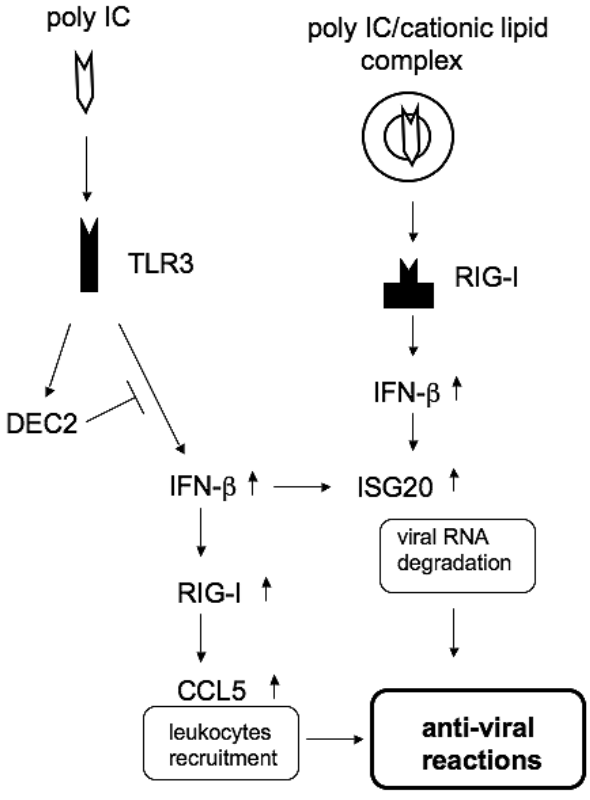

Figure 1 Proposed anti-viral signaling pathways in human mesangial cells.

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