Role of matrix metalloproteinases in viral-associated glomerulonephritis

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Abstract

Background. Viral infections are a major problem worldwide. Many of these infections are frequently complicated by a virus-associated glomerulonephritis. In glomerulonephritis, progression of renal failure is mainly attributed to the development of extensive glomerular and interstitial fibrosis. Advanced glomerular disease is characterized by the accumulation of extracellular matrix components in the mesangial matrix and glomerular basement membrane. These matrix components are metabolized by matrix metalloproteinases (MMPs) as well as tissue inhibitors of metalloproteinase (TIMPs).

Methods. The expression of MMP2, MMP9 and TIMP-1 in human mesangial cells in culture was analysed by RT–PCR.

Results. Mesangial cells express the viral receptors toll-like receptor 3 and RIG-I. Activation of these viral receptors by viral RNA exemplified by poly (I:C) RNA leads to a time- and dose-dependent expression of MMP9 without affecting the expression of MMP2 and TIMP-1. To show the specific effect of viral receptors, knockdown experiments with siRNA specific for TLR3 and RIG-I were performed.

Conclusion. This novel finding of the functional expression of these viral sensors on glomerular fibrosis may indicate a novel link between viral infections and glomerular inflammation and indicates a pathophysiologic role of viral receptors in these processes.

Keywords: MMP-2; MMP-9; RIG-I; TIMP-1; toll-like receptor 3

Introduction

Viral infections are a major problem worldwide. Many of these infections are frequently complicated by a virus-associated glomerulonephritis. During the course of hepatitis C virus infection (HCV), immune complexes and viral RNA reach the mesangium [1,2]. Immunoglobulin A (IgA) nephropathy is the most common glomerulonephritis worldwide and is often preceded by viral upper respiratory tract infections [3]. HIV-associated nephropathy, a disease affecting up to 10% of HIV-seropositive individuals, is a clinical-pathological entity characterized by heavy proteinuria, enlargement of the kidney and rapid progression to renal failure [4]. Glomerulopathy mainly characterized by focal segmental glomerulosclerosis plays a prominent role in the clinical manifestations and progression of HIV-associated nephropathy [4]. In glomerulonephritis, progression of renal failure is mainly attributed to the development of extensive glomerular and interstitial fibrosis [5]. The various types of glomerulonephritis are characterized by inflammatory processes involving a number of factors released by resident and recruited cells, including proinflammatory cytokines and growth factors. The development of glomerular sclerosis and tubulointerstitial fibrosis appears as the common mechanism associated with the loss of renal function and progression towards end-stage renal failure [5,6]. Advanced glomerular disease is characterized by the accumulation of extracellular matrix (ECM) components in the mesangial matrix and glomerular basement membrane [7,8]. These matrix components are metabolized by matrix metalloproteinases (MMPs) as well as tissue inhibitors of metalloproteinase (TIMPs) [9]. The main constituent of mesangial matrix and glomerular basement membrane is type IV collagen that is degraded by MMP2 and MMP9, whereas the degradation of this collagen is mainly inhibited by TIMP-1 [9]. MMP2 synthesis has been shown in fibroblasts and mesangial cells (MCs) while MMP9 is produced by glomerular epithelial cells and MCs [10,11]. MMPs and TIMPs play important roles in various forms of glomerulonephritis [8,9]. These MMPs and TIMPs are also released into the bloodstream, and specific changes in plasma concentrations were found in patients with distinct types of glomerulonephritis [8]. The MMP activities are regulated through pro-enzyme activation and interaction with the TIMPs [12]. The increased expression or activity of MMPs has been described in cases of several different glomerular diseases such as IgA nephropathy [13], membranous glomerulonephritis.
Toll-like receptors (TLRs) are an essential part of the innate immune system. TLRs recognize conserved pathogen-associated molecular patterns (PAMPs) and are expressed not only on immune cells but also on a number of non-immune cells [18]. TLRs recognize molecular patterns associated with microbial pathogens and induce an immune response. Eleven members of the TLR family (TLR1–11) have so far been identified in mice and 10 in humans, each recognizing a distinct component of an infectious agent [19]. TLR3 recognizes dsRNA of viral origin as exemplified by polyriboinosinic-polyribocytidylic acid [poly (I:C) RNA], a synthetic analogue of viral dsRNA [20,21]. TLR3 expression is not only restricted to leukocytes but also occurs in non-immune organs, including lung and bowel [22]. In contrast to most TLRs, signalling of TLR3 is independent of the common TLR adaptor protein MyD88 and involves the adaptor protein Trif (TICAM-1). The identification of the TLR3-TICAM-1 pathway in mammalian cells provides a link between dsRNA and the synthesis of inflammatory cytokines and interferons [23]. Besides TLR3, the helicase retinoic acid-inducible gene 1 (RIG-I) may also act as a sensor of viral infections through recognition of viral dsRNA and may up-regulate type I interferons [24]. We have previously shown a positive staining for TLR3 on MCs, vascular smooth muscle cells and collecting duct epithelium of adult healthy kidney by immunohistochemistry [25]. MCs in cell culture have low TLR3 mRNA levels with a predominant intracellular protein localization that was increased by tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ) and the TLR3 ligand poly (I:C) RNA. Poly (I:C) RNA stimulation of MCs increased mRNA and protein synthesis of IL-6, IL-1β, M-CSF, IL-8/CXCL8, RANTES/CCL5, MCP-1/CCL2 and ICAM-1 and also increased anti-proliferative and proapoptotic effects. We postulated that TLR3 may be important for the clearance of viral RNA reaching the glomerular mesangium, possibly serving in a housekeeping manner under normal conditions. Under pathological conditions such as viral infections, viral RNA alone or as part of immune complexes could reach the mesangium and trigger glomerular inflammation, resulting, e.g. in HCV-associated glomerulonephritis [2,26]. In support of this hypothesis, we found increased mRNA levels for TLR3 and for proinflammatory cytokines and chemokines in microdissected glomeruli from biopsies of hepatitis C associated but not idiopathic membranoproliferative glomerulonephritis and proposed that TLR3 expression in MCs may play a role in some forms of glomerulonephritis. As MMPs play a pivotal role in progression of glomerulonephritis to renal fibrosis and finally end-stage renal disease, we then investigated the effect of viral RNA on mesangial synthesis of MMPs and TIMPs. We postulated that poly (I:C) RNA mimicking viral RNA leads to an increased production of MMP9 without affecting synthesis of MMP2 and TIMP-1. These effects observed are mediated by TLR3 but not by RIG-I.

Methods

Cell culture of human MCs

Immortalized human MCs were grown as described previously [27]. For RNA extraction, MCs were incubated with or without a combination of TNF-α (25 ng/ml), IL-1β (10 ng/ml) and IFN-γ (20 ng/ml) for 24 h, washed with PBS, cultured in the culture medium (containing 10% fetal calf serum) for 6 h and washed again with PBS. Subsequently, MCs were incubated in the culture medium alone (control) or culture medium containing poly (I:C) RNA or poly (I:C) DNA as indicated. Immortalized MCs showed a characteristic appearance of human MCs and did not change their morphology during stimulation experiments. For analysis of mRNA levels, extraction of total RNA was performed using an RNeasy Mini Kit (Qiagen, Germany) with additional DNase digestion.

Quantitative reverse transcriptase–polymerase chain reaction analysis

Quantitative RT–PCR analysis was done as described [28]. For quantitative RT–PCR, 2 μg of isolated total RNA underwent random primed reverse transcription using a modified Moloney murine leukaemia virus reverse transcriptase (Superscript; Life Technologies, Germany). In parallel, 2-μg aliquots were processed without reverse transcription to control for contaminating genomic DNA. Real-time RT–PCR was performed on a TaqMan ABI 7700 sequence detection system (PE Applied Biosystems, Germany). GAPDH was used as reference gene. All water controls were negative for target and housekeeper genes. Sequences, with the following gene bank accession numbers, served for the design of the predeveloped TaqMan assay reagents (PDAR) or primers and probe, purchased from Applied Biosystems: NM_003265/U88879 (human TLR3), NM014314 (human RIG-I), M33197 (human GAPDH), primer sequences: h-MMP2-FP1: TTGAT GGATCGTCTAGATC h-MMP2-RP1: GCTTTGCAGC TTCGCTCA h-MMP2-T1FAM: AGGACCGTGTATT TTGGCCGACTG h-MMP9-FP1: GAGGCGCTCATGTA CCTATGT h-MMP9-RP1: CGCGTGCTAGGCTCGG h-MMP9-T1FAM: ACAGTTGATGGCTCGCCACCTC h-TIMP1-FP1: CTCAGATCCGGTTGCTACAC h-TIMP1-RP1: TGCAAGAGTCACCCTCAGT h-TIMP1-T1FAM: CGCCCATGGAGAGTGTCTGCGGATACT.

Knockdown of gene expression with short interfering RNA

Pre-designed short interfering RNA (siRNA) specific for TLR3 and RIG-I were purchased from Ambion (Japan). Transfection of siRNA into the cells was performed as described before [29]. Scrambled siRNA was used as the non-specific negative control of siRNA (Ambion).

Statistical analysis

Values are provided as mean ± SEM. Statistical analysis was performed by the unpaired t-test. Significant
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Results

Cultured human MCs express the viral receptors TLR3 and RIG-I

As MCs play a role in virus-associated glomerular disease, [25] we examined cultured human MCs for the expression of the viral receptors TLR3 and RIG-I. RNA was prepared from cells growing under standard conditions, as well as from cells that had been stimulated with the cytokines TNF-α, IL-1β and IFN-γ alone or in combination for 24 h to simulate a proinflammatory milieu, as would occur during immune-mediated glomerular disease. Furthermore, MCs were stimulated for 24 h with poly (I:C) RNA (5 µg/ml), a synthetic analogue of viral RNA. By quantitative RT–PCR, specific products for TLR3 and RIG-I mRNA were amplified from both stimulated and unstimulated cells. The expression of TLR3 was increased by each of the cytokines TNF-α, IL-1β and IFN-γ or the combination of these cytokines. Poly (I:C) RNA increased TLR3 expression. The increase in TLR3 expression after poly (I:C) RNA stimulation was equipotent to the effect of the cytokine combination. The basal expression of RIG-I was increased by TNF-α and the cytokine combination. IFN-γ had no effect on the expression of RIG-I. IL-1β increased RIG-I expression, but not significantly. Stimulation of MCs with poly (I:C) RNA increased expression of RIG-I equipotent to the effect of the cytokine combination.

When MCs were incubated with poly (I:C) RNA (5 µg/ml) for up to 24 h, synthesis of TLR3 and RIG-I increased both under basal conditions and further after pretreatment with proinflammatory cytokines. Stimulation of MCs with poly (I:C) RNA (5 µg/ml) led to an increase in synthesis of TLR3 and RIG-I under basal conditions with further enhancement when MCs were pre-treated with TNF-α, IL-1β and IFN-γ. For TLR3, a maximum increase was observed between 12 and 24 h. For RIG-I, the maximum increase was seen between 6 and 12 h. At 12-h poly (I:C) RNA stimulation time, no difference was found between cells stimulated with poly (I:C) RNA with or without cytokine pre-treatment. At 24 h, the highest increase was found under cytokine pre-treated conditions (Figure 1).

Expression of MMPs and TIMP by human MCs

Because MCs play a role in glomerular disease processes and MMPs and its inhibitor TIMPs are key mediators in generation and degradation of ECM components, we examined cultured human MCs for the expression of MMP2, MMP9 and TIMP-1. RNA was prepared from cells growing under standard conditions as well as from cells that had been stimulated with the cytokines TNF-α, IL-1β and IFN-γ alone or in combination for 24 h to simulate a proinflammatory milieu as would occur during immune-mediated glomerular disease. In addition, MCs were stimulated for 24 h with poly (I:C) RNA (5 µg/ml), a synthetic analogue of viral RNA. By quantitative RT–PCR, specific products for MMP2, MMP9 and TIMP-1 mRNA were amplified from both unstimulated and stimulated cells. The basal expression of MMP2 was not affected by stimulation with TNF-α, IL-1β, the cytokine combination (TNF-α, IL-1β, IFN-γ) and poly (I:C) RNA. IFN-γ alone inhibited the expression of MMP2 (Figure 2A). The basal expression of MMP9 could be increased by TNF-α and the cytokine combination. IL-1β and IFN-γ stimulation had no effect on MMP9 expression. Furthermore, poly (I:C) RNA (5 µg/ml) also up-regulated the mRNA of MMP9 (Figure 2B). MCs showed a basal expression of TIMP-1 that was not affected by the cytokine combination, each cytokine alone or by poly (I:C) RNA stimulation (Figure 2C).

Effect of incubation with poly (I:C) RNA on mRNA levels of MMP2, MMP9 and TIMP-1

As MCs exhibit a basal expression of viral receptors TLR3 and RIG-I that could be further increased after stimulation with a combination of proinflammatory cytokines (TNF-α, IL-1β, IFN-γ), MCs were stimulated with poly (I:C) RNA mimicking viral RNA. MCs were incubated with (comb) or without (basal) a combination of cytokines for 24 h, washed with PBS, incubated in culture medium for 6 h and washed again with PBS. Subsequently, MCs were incubated with the culture medium alone (control) or the culture medium containing poly (I:C) RNA in different concentrations (0.5, 5, 10 µg/ml) for 24 h. For MMP2 expression, no increase was observed when the cells were stimulated with different concentrations of poly (I:C) RNA under basal conditions or after additional cytokine pre-treatment. Exposure of MCs maintained under basal conditions to different concentrations of poly (I:C) RNA increased mRNA levels for MMP9 significantly in a dose-dependent manner. As to be expected basal expression for mRNA of MMP9 was increased after stimulation with proinflammatory cytokines. When MCs were pre-treated with the cytokine combination and subsequently stimulated with different concentration of poly (I:C) RNA up to 10 µg/ml, MMP9 expression could be further increased with a peak at a poly (I:C) RNA concentration of 5 µg/ml. The basal expression of TIMP-1 could not be increased when MCs were stimulated with poly (I:C) RNA with or without cytokine pre-treatment (Figure 3).

When MCs were incubated with poly (I:C) RNA (5 µg/ml) for up to 24 h, expression of MMP2 (Figure 4A) and TIMP-1 (Figure 4C) was not affected under basal conditions or when MCs were pre-treated with the combination of proinflammatory cytokines. Production of MMP9 was increased both under basal conditions and further after pretreatment with proinflammatory cytokines with a maximum between 12 and 24 h (Figure 4B). Poly (I:C) DNA (5 µg/ml) did not stimulate any of the MMPs or TIMP-1 both, under basal and cytokine pre-treated conditions (data not shown).

Effect of transfection with siRNA specific for TLR3 and RIG-I on synthesis of poly (I:C) RNA induced MMP and TIMP synthesis

MCs constitutively express mRNA for TLR3 and RIG-I. To test the effectivity of siRNA on gene suppression...
MCs were cultivated under standard conditions for 24 and 48 h. The basal expression of TLR3 and RIG-I was analysed by real-time RT–PCR. Transfection of siRNA specific for TLR3 and RIG-I caused remarkable down-regulation of the basal gene expression of TLR3 (24 h: 93%, 48 h: 89%) and RIG-I (24 h: 89%, 48 h: 79%). Negative control with unspecific RNA had no effect on the gene expression of TLR3 and RIG-I. MCs were stimulated with poly (I:C) RNA (5 µg/ml) for 24 h prior to RNA extraction. To avoid the influence of cytokines on the expression of MMPs and TIMP-1, cells were not pre-treated with the cytokine combination mentioned above. Furthermore, MCs showed a significant reduction in proliferation and an increase in apoptosis when cells were pre-incubated with proinflammatory cytokines and stimulated with poly (I:C) RNA. When MCs were stimulated with poly (I:C) RNA alone without additional cytokine pre-treatment, these effects were only small [25]. Poly (I:C) RNA significantly increased synthesis of MMP9. siRNA specific for TLR3 reduced poly (I:C) RNA-induced mRNA expression of MMP9. siRNA specific for RIG-I had no effect on the poly (I:C) RNA-induced expression of MMP9. As expected, poly (I:C) RNA had no effect on MMP2 and TIMP-1 synthesis. These results were not affected by siRNA specific for TLR3 or RIG-I (data not shown). Negative controls containing unspecific RNA had no effect on the basal expression of MMPs and TIMP-1 or poly (I:C) RNA-induced changes in MMP and TIMP-1 expression (Figure 5).

Discussion

The aim of this study was to show how ECM of the mesangium could be generated in virus-associated forms...
of glomerulonephritis. The results of this study demonstrate that poly (I:C) RNA mimicking viral RNA induces the synthesis of MMP9 in a human MC line without affecting MMP2 and TIMP-1 production. This may represent a potential mechanism of development of matrix abnormalities that are characteristic of immune-mediated renal disease. The development of progressive renal failure in glomerular disease is mainly attributed to the development of extensive glomerular and interstitial fibrosis [5]. The normal function of the renal glomerulus depends on the integrity of the glomerular basement membrane and of the ECM of the mesangium. Fibrosis is characterized by the accumulation of ECM components, which results from the imbalance between an excessive ECM production and a defective ECM degradation by proteolytic enzymes, among which MMPs play a major role [12]. The turnover of ECM components is a tightly controlled equilibrium between synthesis of new matrix constituents and the degradation and removal of old [18]. The main constituent of the mesangial matrix and glomerular basement membrane is type IV collagen that is degraded by MMP2 and MMP9, whereas the degradation of this collagen is mainly inhibited by the tissue inhibitor of MMPs’ TIMP-1 [9]. The secretion of TIMP-1 by MCs is as important as pro-metalloproteinase production in the control of the degradation of the mesangial matrix. MMPs have been implicated in the development of injury in several forms of glomerulonephritis. In mesangial proliferative glomerulonephritis, MMP9 staining was increased mainly in the mesangium and corresponded with the level of glomerular cell proliferation. MMP9 staining was found clearly colocalized with histological markers of active glomerulonephritis, i.e., crescentic glomeruli and within the tubulointerstitial infiltrates [30]. We tested the effect of the cytokines TNF-α, IL-1β, IFN-γ and the combination of these cytokines to simulate a proinflammatory milieu as would occur during immune-mediated glomerular disease on the synthesis of MMP2, MMP9 and TIMP-1 in MCs. TNF-α led to an increase in MMP9 synthesis in our MC line. This result was consistent with earlier observations of increased MMP9 synthesis after TNF-α stimulation in human MCs [17]. The cytokine combination stimulates MMP9 synthesis equipotent to the TNF-α effect. IL-1β or IFN-γ alone had no effect on synthesis of MMP9. IFN-γ alone had a decreasing
Fig. 3. Effect of incubation with poly (I:C) RNA on mRNA levels of MMP2, MMP9 and TIMP-1. MCs were pre-incubated with (comb) or without (basal) a combination of proinflammatory cytokines (TNF-α, IL-1β, IFN-γ) for 24 h, washed with PBS, incubated in culture medium for 6 h, washed again with PBS and incubated with or without (control) poly (I:C) RNA in different concentrations (0.5, 5, 10 µg/ml) for 24 h before RNA extraction.

Levels of mRNA expression for MMP2, MMP9 and TIMP-1 were analysed by real-time RT–PCR. (A) Expression of MMP2 was not affected by poly (I:C) RNA under basal conditions or after additional cytokine pre-treatment. (B) Stimulation with poly (I:C) RNA increased MMP9 expression of MCs in a concentration-dependent manner in a range from 0.5 to 10 µg/ml poly (I:C) RNA, an effect enhanced by pre-treatment with the cytokine combination with a peak at 5 µg/ml poly (I:C) RNA. (C) No effect on basal TIMP-1 expression was seen when the cells were stimulated with different concentration of poly (I:C) RNA with or without cytokine pre-treatment. Results are mean ± SEM of two independently performed experiments and rRNA served as the reference gene.

effect on MMP2 production, but TNF-α or IL-1β alone as well as the combination of these three cytokines had no effect on basal MMP2 synthesis. Basal TIMP-1 synthesis was not affected by the cytokines alone or the cytokine combination.

TLR3 and RIG-I are known as receptors for dsRNA. Viral dsRNA has been recognized as a major ligand for TLR3 in many cell types including DCs and non-immune cells such as intestinal epithelial cells, fibroblasts and MCs [25,31]. This is considered to serve as an anti-viral system by generating type I interferons. It has been recently shown that single-stranded RNA can also act as a ligand for TLR3. Polyriboinosinic:polyribocytidylic acid is a synthetic analogue for viral RNA and activates TLR3 [32]. In addition to TLR3, a role for RIG-I in viral infections was shown for several cell types [24,29].

We have previously shown a role for TLR3 in hepatitis C-associated glomerulonephritis [25]. Adult healthy kidney showed a positive staining for TLR3 on MCs, vascular smooth muscle cells and collecting duct epithelium. MCs in cell culture have low TLR3 mRNA levels with a predominant intracellular protein localization that was increased by TNF-α, IL-1β, IFN-γ, the combination of these cytokines and the TLR3 ligand poly (I:C) RNA. Poly (I:C) RNA stimulation of MCs increased mRNA and protein synthesis of IL-6, IL-1β, M-CSF, IL-8/CXCL8, RANTES/CCL5, MCP-1/CCL2 and ICAM-1 and also increased anti-proliferative and proapoptotic effects. TLR3 might be important for the clearance of viral RNA reaching the glomerular mesangium, possibly serving in a housekeeping manner under normal conditions. Under pathological conditions such as viral infections, viral RNA alone or as part of immune complexes could reach the mesangium and trigger glomerular inflammation, resulting e.g. in HCV-associated glomerulonephritis. In support of this hypothesis, we found increased mRNA levels for TLR3 and for proinflammatory cytokines and chemokines in microdissected glomeruli from biopsies of hepatitis C-associated but not idiopathic membranoproliferative glomerulonephritis and proposed that TLR3 expression in MCs may play a role in some forms of glomerulonephritis.

In the present study we stimulated MCs with poly (I:C) RNA mimicking viral RNA and analysed mesangial...
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Fig. 4. Time-dependent influence of poly (I:C) RNA on synthesis of MMPs and TIMP-1. MCs were pre-treated under basal (open symbols) or cytokine combination conditions (filled symbols) as described in the Methods section and after washing they were incubated with (triangles, discontinued line) or without (squares, continuous line) poly (I:C) RNA (5 μg/ml) for different time intervals (3, 6, 12 or 24 h). The expressions of MMP2 (A), MMP9 (B) and TIMP-1 (C) were determined by real-time RT-PCR. Incubation of MC with poly (I:C) RNA had no effect on synthesis of MMP2 and TIMP-1. Poly (I:C) RNA stimulation results in a concentration-dependent increase in MMP9, an effect enhanced by pre-treatment with the cytokine combination. Poly (I:C) DNA (5 μg/ml) had no effect on MMP and TIMP-1 mRNA levels (data not shown). Values are means ± SEM of two independently performed series of experiments.

expression of MMP2, MMP9 and TIMP-1 by real-time RT-PCR. Incubation of MCs with poly (I:C) RNA under basal conditions resulted in an up-regulation of MMP9. This effect was dose and time dependent. When MCs were pre-treated with the cytokine combination, this effect could be further increased. These results might be explained by an additional stimulating effect of proinflammatory cytokines and poly (I:C) RNA or by an increasing effect mediated by viral sensors, which are up-regulated after cytokine stimulation [25]. Nee et al. showed that MMP2 production was unaffected by TNF-α or IL-1β [17], a result that was reproducible in our cell line. In addition, poly (I:C) RNA had no effect on MMP2 expression under basal conditions or after additional cytokine pre-treatment (present result). This finding is consistent with the view that MMP2 expression tends to be constitutive and is thought to perform a surveillance function [33]. The basal expression of TIMP-1 was not affected by poly (I:C) RNA with or without cytokine pre-treatment. We studied the specific roles of the viral receptors TLR3 and RIG-I by using knockdown experiments with siRNA specific for each receptor. The poly (I:C) RNA-mediated expression of MMP9 was inhibited by siRNA specific for TLR3 but not for RIG-I. These results indicate that the effect of viral RNA on increased mesangial MMP9 synthesis is mainly mediated by TLR3. As expected siRNA specific for TLR3 or RIG-I had no effect on MMP2 and TIMP-1 synthesis. Poly (I:C) DNA did not stimulate any of the MMPs or TIMP-1 arguing in favour of a TLR3-mediated effect by poly (I:C) RNA.

Our results demonstrate how viral RNA exemplified by poly (I:C) RNA might influence the process of generation and degradation of mesangial ECM during immune-mediated glomerulonephritis. In this context, it is interesting that MMPs play an important role in hepatitis C-induced fibrosis and cirrhosis of the liver or even pathogenesis of hepatitis C-related hepatocellular carcinoma [34,35]. Although our results only show an association, we consider this a novel and attractive hypothesis for viral disease-associated glomerulonephritis or even for viral disease-triggered exacerbation of other forms of glomerular injury, e.g. lupus erythematosus. The latter hypothesis is supported by data showing a marked worsening of the chronic lupus-like nephritis of MRL-Fas(lpr) mice injected with poly (I:C) RNA [36].

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Conflict of interest statement. None declared.
Fig. 5. Effects of transfection with siRNA for TLR3 and RIG-I on poly (I:C) RNA-induced expression of MMPs and TIMP-1. To test the effectivity of siRNA on gene suppression the basal expression of TLR3 (A) and RIG-I (B) was analysed at different time points (24, 48 h) by real-time RT–PCR. siRNA specific for TLR3 and RIG-I suppressed the basal gene expression significantly. Negative control with unspecific RNA had no effect on the gene expression of TLR3 and RIG-I. (C) MCs were stimulated with poly (I:C) RNA (5 µg/ml) for 24 h. siRNA specific for TLR3 clearly reduced the poly (I:C) RNA-induced mRNA expression of MMP9. siRNA specific for RIG-I had no effect on the poly (I:C) RNA-induced expression of MMP9. Poly (I:C) RNA had no effect on basal MMP2 and TIMP-1 synthesis. These results were not affected by siRNA specific for TLR3 or RIG-I (data not shown). Values are means ± SEM of two independently performed series of experiments.

References

Expression of gremlin and vascular calcification

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Abstract

Background. Vascular calcification has been widely recognized as a significant contributor to cardiovascular risk in patients with chronic kidney disease. Recent evidence suggests that BMP-7 decreases the vascular calcification observed in uraemic rats, while BMP-2 could also be participating in this process. Gremlin, a bone morphogenetic protein antagonist, has been detected in rat aortic vascular smooth muscle cells (VSMCs), and since the role

of the VSMCs into vascular calcification in uraemia is considered critical in this process, we hypothesized that gremlin could be participating in its pathogenesis. With this aim, we studied its expression in aorta from uraemic rats with calcitriol-induced vascular calcification and in 16-vessel biopsies of uraemic patients undergoing kidney transplantation.

Methods. Gremlin was detected by in situ hybridization (ISH) and immunohistochemistry (IMH). BMP-7, BMP-2 and BMP-2 receptor (BMPR2) were detected by IMH. Vascular calcification was assessed by von Kossa staining method. Sham-operated and 5/6 nephrectomized rats (NFR) (1.2%) were treated with vehicle or calcitriol (80 ng/kg, intraperitoneally every other day). Rats were

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