Ribosomal protein S19 is a novel therapeutic agent in inflammatory kidney disease

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Abstract

RPS19 (ribosomal protein S19), a component of the 40S small ribosomal subunit, has recently been identified to bind the pro-inflammatory cytokine macrophage MIF (migration inhibitory factor). In vitro experiments identify RPS19 as the first endogenous MIF inhibitor by blocking the binding of MIF to its receptor CD74 and MIF functions on monocyte adherence to endothelial cells. In the present study, we sought to establish whether recombinant RPS19 can exert anti-inflammatory effects in a mouse model of anti-GBM (glomerular basement membrane) GN (glomerulonephritis) in which MIF is known to play an important role. Accelerated anti-GBM GN was induced in C57BL/6J mice by immunization with sheep IgG followed 5 days later by administration of sheep anti-mouse GBM serum. Groups of eight mice were treated once daily by intraperitoneal injection with 6 mg of RPS19/kg of body weight or an irrelevant control protein (human secretoglobin 2A1), or received no treatment, from day 0 until being killed on day 10. Mice that received control or no treatment developed severe crescentic anti-GBM disease on day 10 with increased serum creatinine, declined creatinine clearance and increased proteinuria. These changes were associated with up-regulation of MIF and its receptor CD74 activation of ERK (extracellular-signal-regulated kinase) and NF-κB (nuclear factor κB) signalling, prominent macrophage and T-cell infiltration, as well as up-regulation of Th1 [T-bet and IFNγ (interferon γ)] and Th17 [STAT3 (signal transducer and activator of transcription 3) and IL-17A] as well as IL-1β and TNFα (tumour necrosis factor α). In contrast, RPS19 treatment largely prevented the development of glomerular crescents and glomerular necrosis, and prevented renal dysfunction and proteinuria (all \( \text{P} \leq 0.001 \)). Of note, RPS19 blocked up-regulation of MIF and CD74 and inactivated ERK and NF-κB signalling, thereby inhibiting macrophage and T-cell infiltration, Th1 and Th17 responses and up-regulation of pro-inflammatory cytokines (all \( \text{P} < 0.01 \)). These results demonstrate that RPS19 is a potent anti-inflammatory agent, which appears to work primarily by inhibiting MIF signalling.

Key words: kidney disease, macrophage, migration inhibitory factor (MIF) inhibitor, ribosomal protein S19 (RPS19), T-cell

INTRODUCTION

It is now apparent that ribosomal proteins have many extraribosomal activities. Ribosomal proteins from micro-organisms to mammals have been shown to regulate a wide variety of functions including chromatin structure, gene transcription, RNA processing and splicing and post-translational modifications [1]. RPS19 (ribosomal protein S19) is a 16 kDa protein, which is best known as a component of the 40S ribosomal subunit. However, unlike most other ribosomal proteins, RPS19 is also found in the plasma, suggesting novel extracellular functions for this protein. Indeed, RPS19 is present as a complex with prothrombin in the blood stream [2]. Proteomic analysis has identified many proteins capable of binding to immobilized recombinant RPS19 [3]. Of particular interest, RPS19 was recently identified as a binding protein for macrophage MIF (migration inhibitory factor) [4]. Low doses of RPS19 were found to strongly inhibit MIF binding to its receptor CD74. Furthermore, RPS19 significantly reduced CXCR2 (CXC chemokine receptor 2)-dependent MIF-triggered adhesion of monocytes to endothelial
cells under flow conditions in vitro [4]. However, the hypothesized role for RPS19 to inhibit MIF activity in vivo has not been examined. MIF is a pro-inflammatory cytokine and plays a pathogenic role in a variety of inflammatory diseases as shown by studies using MIF gene-deficient mice, neutralizing antibodies and small MIF antagonists [5–7]. Thus RPS19 may be an important regulator of MIF function in normal and diseased states. MIF is highly up-regulated in both human and animal models of GN (glomerulonephritis) [7–11]. To examine whether RPS19 has potential as an anti-inflammatory therapeutic, we used a model of severe inflammatory renal injury in which we have previously established MIF to play a critical pathogenic role using a neutralizing antibody [7]. Therefore we investigated the effects of daily administration of recombinant RPS19 in a mouse model of accelerated anti-GBM (glomerular basement membrane) GN in which macrophages and T-cells cause severe renal damage [6,7–15].

MATERIALS AND METHODS

Reagents
Recombinant RPS19 protein and an irrelevant control protein, human secretoglobin SCGB 2A1, were produced in Escherichia coli as described previously [4]. SCGB 2A1 was selected as an irrelevant control protein on the basis of its similar molecular mass to RPS19, its lack of enzymatic activity and being a secreted protein present in the extracellular space.

Mouse model of anti-GBM GN
Accelerated anti-GBM GN was induced in C57BL/6J mice (25 g; age, 8 weeks) following an established protocol [16]. C57BL/6J mice were immunized with sheep IgG in Freund’s complete adjuvant followed 5 days later by administration of sheep anti-mouse GBM immunoglobulin at a dose of 60 μg/g of body weight (term day 0). We first performed a pilot study in which accelerated anti-GBM disease was induced in groups of three mice which received once daily intraperitoneal injections of recombinant RPS19 in saline at 2.5, 5 or 10 mg/kg of body weight beginning at day 0 and continuing until mice were killed 14 days later. After an optimal dose of RPS19 had been identified, groups of eight mice were treated once daily with 6 mg of RPS19/kg of body weight or control SCGB 2A1 (6 mg/kg of body weight), or not treated at all from day 0 until being killed on day 10. A group of normal C57BL/6J mice was used as normal control. Kidney tissue was collected for histology, immunohistochemistry and real-time PCR analysis. The experimental procedures were approved by the Committee on the Use of Live Animals for Teaching and Research at the Chinese University of Hong Kong.

Measurement of proteinuria and ELISA
All mice were housed overnight in metabolic cages for 16 h urine collections before disease induction and on days 0 and 1, 4 and 5 and 9 and 10 of anti-GBM disease. Urinary protein excretion was determined using the Coomassie Brilliant Blue method [14,16]. Plasma and urinary creatinine levels were measured by ELISA following established protocols [14,16].

Real-time PCR
Total kidney RNA was extracted using the RNeasy Kit according to the manufacturer’s instructions (Qiagen). cDNA was synthesized and real-time PCR was run on an Opticon 2 real-time PCR machine (Bio-Rad) using the IQ SYBR Green supermix reagent (Bio-Rad) as described previously [16]. Primers for mouse MIF, CD74, IL-1β, TNFα (tumour necrosis factor α), MCP-1 (monocyte chemoattractant protein 1), IFNγ (interferon γ), T-bet and GAPDH (glyceraldehyde-3-phosphate anti-sheep immunoglobulins were measured by ELISA following established protocols [14,16].

Figure 1 Pilot study of RPS19 in mouse anti-GBM disease (A) Urinary protein excretion (μg/16 h). (B–E) PAS-stained sections show that the untreated mice exhibit severe glomerular damage with crescent formation and tubulointerstitial damage including cast formation in many dilated tubules, which is inhibited by RPS19 (S19) in a dosage-dependent manner with optimal effects at doses of 5 and 10 mg/kg of body weight. (F–H) Quantification of glomerular crescent formation and infiltration of CD3+ T-cells and F4/80+ macrophages in the kidney cortex show substantial protection from histological damage. Data represent means ± S.E.M. for a group of three mice. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with day 0; #P < 0.05, ##P < 0.01 and ###P < 0.001 compared with the untreated mice.
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Figure 2  Effect of RPS19 treatment on histology and renal function

(A) Representative histology sections stained with PAS. Compared with normal mouse kidney (NR), severe glomerular crescent formation, segmental glomerular necrosis and tubulointerstitial damage are evident on day 10 of anti-GBM disease in mice given either no treatment (GN) or animals treated with an irrelevant control protein (SCGB 2A1). By contrast, RPS19 (S19) treatment substantially ameliorated histological damage in anti-GBM disease. (B) Urinary protein excretion. (C) Serum creatinine. (D) Creatinine clearance. Each bar represents means ± S.E.M. for groups of eight mice.

*P < 0.05, **P < 0.01 and ***P < 0.001 compared with normal mice; #P < 0.05, ##P < 0.01 and ###P < 0.001 compared with the untreated anti-GBM GN. Original magnification × 200 (A).

Dehydrogenase) have been described previously [16,17]. Other primers used in the present study are as follows: IL-17A forward, 5′-GCTCCAGAAGGCCCTCAGA-3′, reverse, 5′-AGCTTTCCCTCCGATTA-3′; STAT3 (signal transducer and activator of transcription 3) forward, 5′-CAATACCATTGACCCTGCCGAT-3′, reverse, 5′-GAGCGACTCAAATGCCCT-3′.

Western blot analysis
Protein from kidney tissues was extracted using a RIPA lysis buffer, and Western blot analysis was performed as described previously [16]. After blocking non-specific binding with 5% (w/v) BSA, membranes were incubated overnight at 4°C with the primary antibodies against phospho-ERK1/2 (extracellular-signal-regulated kinase1/2) MAPK (mitogen-activated protein kinase), total ERK1/2 MAPK or GAPDH (Chemicon), followed by IRDye800-conjugated secondary antibody (Rockland Immunochemicals). Signals were detected using the Odyssey IR imaging system (LI-COR Biosciences). Quantitative analysis of images was performed using Image J software (National Institutes of Health).

Histology and immunohistochemistry
Changes in renal morphology were examined in methyl Carnoy’s-fixed, paraffin-embedded tissue sections (4 μm) stained with PAS (periodate–Schiff) reagent. Immunostaining was performed in paraffin sections using a microwave-based antigen retrieval technique [16,17]. The antibodies used in the present study included: SP7 rabbit polyclonal anti-CD3 antibody (Abcam), F4/80 rat anti-mouse monoclonal antibody (Serotec); rabbit polyclonal antibodies to IL-1β, MCP-1, PCNA (proliferating-cell nuclear antigen), MIF and goat polyclonal antibodies to TNFα and CD74 (Santa Cruz Biotechnology); and a rabbit polyclonal antibody to phospho-p65 NF-κB (nuclear factor κB) subunit (Cell Signaling Technology).

Immunofluorescence microscopy
Glomerular deposition of immune reactants was assessed by direct immunofluorescence using FITC-conjugated polyclonal antibodies to sheep IgG, mouse IgG and complement C3 as described previously [14,16]. Semi-quantification of sheep IgG, mouse IgG and complement C3 staining was performed using an antibody titration method [7,16].

Quantification of renal pathology and immunostaining
The percentage of glomeruli exhibiting crescent formation and necrosis was scored by counting 50 glomeruli per animal on PAS-stained sections. The number of positive cells stained with CD3, F4/80, PCNA and phospho-p65 NF-κB antibodies were counted in 20 consecutive glomeruli and expressed as cells/gcs.
Figure 3  Effect of RPS19 treatment on glomerular immune deposits

(A) Representative immunofluorescence staining of glomeruli from mice with anti-GBM disease given no treatment, the irrelevant control protein SCGB 2A1 (SCGB) or RPS19 (S19) for the deposition of sheep IgG, mouse IgG and mouse C3. (B–D) Histograms show semi-quantification of glomerular deposition of sheep IgG (B), mouse IgG (C) and mouse C3 (D). Note that although there is no significant difference in the deposition of either sheep anti-GBM immunoglobulin or mouse IgG, there is a partial reduction in glomerular C3 deposition. (E) ELISA shows that there is no difference in the plasma concentration of mouse anti-sheep immunoglobulins among the three groups. Each bar represents means ± S.E.M. for groups of eight mice. ### P < 0.001 compared with the untreated anti-GBM GN group. Original magnification ×400 (A).

(glomerular cross-section), while the positive cells in the cortical tubulointerstitium were counted in 20 consecutive high-power fields (magnification ×400) by means of a 0.0625 mm² graticule fitted in the eyepiece of the microscope and expressed as cells per mm². All scoring was performed on the blinded slides.

Statistical analysis

Results are expressed as the means ± S.E.M. Statistical analyses were performed using one-way ANOVA with a Newman–Keuls post-test from Prism 5.0 GraphPad Software.

RESULTS

Pilot study of RPS19 in mouse anti-GBM disease

We performed a pilot study to determine the optimal dose of RPS19 for the treatment of anti-GBM disease. Groups of four mice received once daily administration of recombinant RPS19 at 2.5, 5 or 10 mg/kg of body weight, beginning on day 0 (when anti-GBM serum was administered) and continued until mice were killed 14 days later. Untreated mice developed proteinuria and severe renal pathology featuring glomerular crescent formation and tubulointerstitial damage, with a prominent infiltrate of F4/80+ macrophages and CD3+ T-cells (Figure 1). The
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Figure 4 Effect of RPS19 treatment on immune cell infiltration and cell proliferation in anti-GBM GN

(A) F4/80+ macrophage infiltration; (B) CD3+ T-cell infiltration; and (C) PCNA+ proliferating cells. Compared with normal kidney (NR), immunohistochemistry shows a marked infiltration of F4/80+ macrophages, CD3+ T-cells or PCNA+ proliferating cells in untreated mice with anti-GBM GN and in diseased animals injected with the irrelevant protein SCGB 2A1 (SCGB). In contrast, these inflammatory and proliferative responses are largely inhibited by RPS19 treatment (S19). (i) Normal, (ii) anti-GBM GN, (iii) anti-GBM GN treated with control SCGB, (iv) anti-GBM GN treated with RPS19, (v, vi) quantification of the immunohistochemical staining sections. Each bar represents the means ± S.E.M. for groups of eight mice.

* P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the normal mice; # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the untreated anti-GBM GN mice. Original magnification ×200 (NR, GN, SCGB and S19).

2.5 mg/kg of body weight dose of RPS19 had little effect on disease, but RPS19 at both 5 and 10 mg/kg of body weight resulted in marked protection from proteinuria, glomerular and tubulointerstitial damage and leucocyte infiltration (Figure 1). On the basis of these data, we used a dose of RPS19 of 6 mg/kg of body weight for studying the therapeutic effect of RPS19 on anti-GBM GN.

RPS19 prevents renal functional and histological injury in anti-GBM disease

The induction of accelerated anti-GBM disease in mice injected with an irrelevant control protein or were left untreated, resulted in severe renal injury featuring glomerular crescent formation, glomerular necrosis and marked tubulointerstitial damage with prominent mononuclear cell infiltration (Figure 2A). These animals also exhibited significant proteinuria and a loss of renal function on the basis of an increased serum creatinine and a reduction in creatinine clearance [CCR (creatinine clearance rate)] (Figures 2B–2D). RPS19 treatment markedly improved renal morphology, with an 80% reduction in glomerular crescent formation and glomerular necrosis, while mononuclear cell infiltration and tubulointerstitial damage was largely prevented (Figure 2A). In addition, RPS19 treatment prevented a loss of renal function and significantly reduced proteinuria (Figures 2B–2D).
RPS19 inhibits up-regulation of inflammatory cytokines in anti-GBM disease

Pro-inflammatory cytokines IL-1 and TNFα are important mediators of renal injury in anti-GBM disease, and their up-regulation in kidney is dependent upon macrophage infiltration [7,8,11,18,19], whereas MCP-1/CCL2 (CC chemokine ligand 2) plays an important role in renal macrophage infiltration in this model [12,13]. Diseased mice left untreated and injected with control protein showed a marked up-regulation of IL-1β, TNFα and MCP-1 expression both in mRNA levels by real-time PCR and protein expression by immunohistochemistry (Figure 6). In contrast, RPS19 treatment largely abrogated these pro-inflammatory responses in the diseased kidney (Figure 6).

RPS19 does not affect the antibody response in anti-GBM disease

Immunofluorescence staining and quantification showed no difference in the prominent glomerular deposition of sheep anti-GBM antibody or mouse IgG among all groups (Figures 3A–3C). Consistent with these data, serum levels of mouse IgG specific for sheep immunoglobulin were equivalent in all groups, indicating no effect of RPS19 on the systemic immune response (Figure 3E). However, the glomerular deposition of complement component C3 seen in untreated and SCGB-treated mice was partially reduced by RPS19 treatment (Figure 3D).

RPS19 inhibits leucocyte infiltration, cell proliferation and T-cell activation in anti-GBM disease

Induction of anti-GBM disease in untreated mice and mice treated with control protein resulted in prominent renal infiltration of F4/80+ macrophages and CD3+ T-cells (Figures 4A and 4B). These mice also showed a marked increase in cell proliferation in glomeruli (particularly within crescents) and in damaged tubules and interstitial cells as shown by PCNA immunostaining (Figure 4C). Interestingly, an increase in renal mRNA levels of molecules involved in the Th1 response (T-bet and IFNγ) and the Th17 T-cell response (STAT3 and IL-17A) was also demonstrated in the kidney of anti-GBM disease (Figure 5), indicating that both Th1 and Th17 immune responses mediate crescentic GN.

RPS19 treatment essentially abolished the macrophage and T-cell infiltrates in anti-GBM disease (Figures 4A and 4B). Consistent with the protection from renal damage seen in PAS-stained sections and the reduction in leucocyte infiltration, RPS19 treatment also substantially reduced glomerular, tubular and interstitial cell proliferation (Figure 4C). Moreover, RPS19 treatment also blunted the Th1 and Th17 immune responses in the kidney as seen by suppressing mRNA levels of T-bet/IFNγ and STAT3/IL-17A expression (Figure 5).

RPS19 inhibits activation of ERK and NF-κB signal pathways

It is reported that MIF acts by stimulating the downstream mediator ERK and NF-κB pathways to regulate cell proliferation and inflammatory responses [20–24]. In the present study, we also found that a substantial increase in ERK signalling, as indicated by ERK phosphorylation, was seen in untreated and control protein treated anti-GBM disease (Figure 8A). Activation of NF-κB signalling, as indicated by nuclear translocation of the phosphorylated p65 subunit, in glomeruli and the tubulointerstitium was also evident in these groups (Figure 8B). Consistent with the marked reduction in cell proliferation, RPS19 treatment substantially reduced ERK phosphorylation (Figure 8A). Similarly, consistent with the reduction in renal inflammation, RPS19 treatment reduced nuclear staining of the phosphorylated NF-κB subunit p65 (Figure 8B).
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Figure 6  Effect of RPS19 treatment on renal expression of pro-inflammatory cytokines and chemokines in anti-GBM GN
(A) IL-1β expression; (B) TNFα expression and (C) MCP-1 expression. Compared with the normal kidney (NR), immunohistochemistry shows a marked infiltration of up-regulation of IL-1β, TNFα and MCP-1 protein expression in the untreated mice (GN) and those given an irrelevant protein (SCGB) in anti-GBM GN, which is largely prevented by RPS19 treatment (S19). (i) Normal, (ii) anti-GBM GN, (iii) anti-GBM GN treated with control SCGB, (iv) anti-GBM GN treated with RPS19, (v) quantification of the immunohistochemistry, (vi) quantification of the mRNA expression by real-time PCR. Each bar represents the means ± S.E.M. for groups of eight mice. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with the normal mice; #P < 0.05, ###P < 0.001 compared with the untreated anti-GBM GN mice. Original magnification, ×200 (A–C).

DISCUSSION

The present study has identified a profound immunosuppressive action of RPS19 in a mouse model of crescentic anti-GBM disease. The potential mechanisms underlying this novel finding are considered below. RPS19 prevented severe kidney damage and loss of renal function by blocking the inflammatory response in anti-GBM disease. The protective effect of RPS19 on anti-GBM GN was associated with a suppressive effect on the cellular immune response by blocking macrophage and T-cell infiltration to the kidney and Th1 and Th17 immune reactions, despite the glomerular deposition of humoral immune reactants and a pre-existing systemic immune response to sheep IgG. In addition, up-regulation of the classic pro-inflammatory cytokines IL-1β and TNFα was prevented by RPS19. This may be related to a direct effect of RPS19 or simply by prevention of macrophage infiltration since macrophages are required for initiating up-regulation of these cytokines within the inflamed kidney [10–13].
inhibitory effect of RPS19 on anti-GBM crescentic GN may be attributed largely to inhibition of MIF signalling such as up-regulation of MIF and its receptor CD74. Although it is difficult to dissect the precise mechanisms of action of RPS19 in this disease model, it is reasonable to postulate that inhibition of MIF activity may be the primary mechanism by which RPS19 inhibited anti-GBM disease based upon the following evidence. First, in vitro studies have established that RPS19 can bind to MIF and even very low concentrations of RPS19 can inhibit MIF binding to its receptor CD74 [4]. Furthermore, RPS19 can inhibit CXCR2-dependent MIF-triggered adhesion of monocytes to endothelial cells under flow conditions indicating a functional role for RPS19 in blocking MIF bioactivity [4]. The present study added new information that addition of RPS19 was capable of blocking MIF signalling by suppressing MIF and CD74 expression in vivo in anti-GBM crescentic GN. Thus blockade of the MIF–CD74 interaction may be a key mechanism by which RPS19 may exert its immunosuppressive effect on immune-mediated injury.

Secondly, the inhibitory effects of RPS19 on anti-GBM GN may be largely attributed to suppress the inflammatory response of MIF, including macrophage and T-cell infiltration and up-regulation of pro-inflammatory cytokines. The effect of RPS19 treatment is entirely consistent with the immunosuppressive effects of administration of a neutralizing anti-MIF antibody in a rat model of accelerated anti-GBM disease in which neutralizing anti-MIF antibody suppressed crescent GN by blocking macrophage and T-cell infiltration and the up-regulation of IL-1β and TNFα, and MIF itself [7,19]. In addition, the use of the MIF small-molecule inhibitor ISO-1 [(S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester], that binds to the tautomerase active site of MIF [25], also produced a similar immunosuppressive action in a mouse models of lupus nephritis [26]. Blockade of an inflammatory response with ISO-1 has also been reported in a number of disease models including autoimmune diabetes [27], experimental sepsis and autoimmune encephalomyelitis [28,29]. Taken together, RPS19, similar to ISO-1, may prevent anti-GBM GN by inhibiting renal inflammation.

Thirdly, inhibition of a Th1 and Th17 response could represent an additional mechanism by which RPS19 suppresses anti-GBM GN. It is now well accepted that T-cell activation plays a critical role in anti-GBM crescentic GN [14,33]. MIF also plays a regulatory role in T-cell activation and IL-17A expression [30–32]. Consistent with these findings, the present study demonstrated that untreated and control protein-treated mice with anti-GBM disease developed a substantial glomerular and...
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Figure 8 Effect of RPS19 on activation of ERK and NF-κB pathways

(A) Western blot analysis shows an increase in ERK phosphorylation in the untreated and SCGB control protein-treated anti-GBM GN, which was significantly inhibited by RPS19 (S19) treatment. (B) Immunohistochemical analysis shows that compared with the normal mice (NR), a marked increase in the number of cells with nuclear localization of the phosphorylated p65 subunit of NF-κB is visible in the diseased kidney received no treatment (GN) or control protein (SCGB), which is blocked by RPS19 treatment. (i) Normal, (ii) anti-GBM GN, (iii) anti-GBM GN treated with control SCGB, (iv) anti-GBM GN treated with RPS19, (v, vi) quantification of the immunohistochemical staining sections. Each bar represents the means ± S.E.M. for groups of eight mice. **P < 0.01 and ***P < 0.001 compared with the normal mice; ##P < 0.05 and ###P < 0.001 compared with the untreated anti-GBM GN. Original magnification, ×200 (B).

It should be pointed out that in the present study RPS19 was administered at the same time as anti-GBM GN was induced. Thus results from the present study may provide evidence for the preventive effect of RPS19 on anti-GBM GN. To prove the therapeutic potential clinically, treatment with RPS19 after the onset of disease is warranted.

In conclusion, we have established that RPS19 is a potent immunosuppressive molecule in experimental renal kidney disease. The results of the present study suggest that the major action of RPS19 is to inhibit both immunological and pro-inflammatory functions of MIF by blocking the MIF–CD74 signalling pathway. However, we cannot rule out other potential mechanisms for the profound effects of RPS19 treatment. Further studies are needed to delineate the precise mechanism of the immunosuppressive action of RPS19 and to establish its potential in other inflammatory diseases.

CLINICAL PERSPECTIVES

- MIF plays a critical role in many inflammatory and autoimmune diseases including the kidney disease.
- The results of the present study demonstrate that recombinant RPS19 acts as a MIF inhibitor capable of blocking MIF signalling and inhibiting macrophage and T-cell infiltration and activation, resulting in the suppression of progressive renal injury in anti-GBM crescentic GN.
- These finding suggest that RPS19 may be a novel therapeutic agent for inflammatory diseases.
AUTHOR CONTRIBUTION

Jun Lv and Xiao Ru Huang performed the experiments, analysed the data and wrote the paper. Suada Fröhlich, Philipp Lacher, Jörg Klug and Andreas Meinhardt generated the purified recombinant RPS19 and SCGB 2A1, and edited the paper. Anping Xu revised the paper. Hui Yao Lan designed the experiments and revised the paper.

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