Circulating IgA1 Complex Induces Endothelial Injury through Mesangial Cell Activation in IgA Nephropathy

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Abstract

Background: Renal arteriolar microangiopathic (MA) lesions were common in IgA nephropathy (IgAN) and their presence was an independent risk factor for kidney failure. Endothelial injury is regarded as the most important cause of MA lesions. The deposition of circulating IgA1 complex (cIgA1) in the glomerular mesangium has been regarded as the most important mechanism to induce the injury of podocyte and tubular epithelium. Whether cIgA1 can induce vascular endothelial injury is not clear.

Methods: Blood of 59 IgAN patients and 19 healthy controls was collected, pending the measurement of Willebrand factor (vWF) and soluble vascular cell adhesion molecule 1 (sVCAM1) and Heparin-binding EGF-like growth factor (HB-EGF) by ELISA. cIgA1 was isolated from 12 primary IgAN patients who had not been treated with steroids or immunosuppressants and 10 healthy controls. Then, IgA-HMC medium (IgA-HMCM) was prepared by collecting the cell culture supernatants of growth-arrested HMCs grown in medium containing cIgA1. Human umbilical vein endothelial cells (HUVECs) were cultured with the IgA-HMCM in the presence or absence of Panax notoginseng saponins (PNS), which are anti-inflammatory bioactive components of the Chinese medicinal herb Panax notoginseng. The protein levels of interleukin-6 (IL-6) and chemokine (C-X-C motif) ligand 1 (CXCL1) in IgA-HMCM and vWF, sVCAM1 and HB-EGF in HUVEC medium were determined by ELISA.

Results: IgAN patients had higher levels of vWF, sVCAM-1 and HB-EGF than that in healthy controls. cIgA1 from IgAN patients (IgAN-cIgA1) induced significantly higher expression levels of IL-6 and CXCL1 in HMCM than cIgA1 from healthy controls. vWF, sVCAM1 and HB-EGF levels induced by conditioned IgAN-cIgA1-treated HMCM (IgAN-HMCM) were significantly higher than the levels induced by conditioned cIgA1-treated HMCM from healthy controls (HC-HMCM). After HUVECs were cultured in PNS and IgAN-HMCM, the levels of vWF, sVCAM1 and HB-EGF were significantly lower in the culture supernatants of cells cultured in PNS and IgAN-HMCM than in that of cells cultured in IgAN-HMCM alone.

Conclusion: cIgA1 could activate mesangial cells and, in turn, induce the injury of endothelial cells by inflammation factors *in vitro*.

Background

IgA nephropathy (IgAN) is the most common primary glomerulonephritis, and 30–40% of IgAN patients progress to end-stage renal disease within 20–30 years[1, 2]. The pathological lesions in IgAN are very complex, including mescangial cell proliferation, podocyte hypercellularity, tubular and interstitial fibrosis. Intrarenal arterial and arteriolar lesions are very common pathological features of IgAN, most of which are associated with hypertension. Microangiopathy (MA) is characterized by endothelial swelling, subintimal edema and narrowing of the lumen, which is not rare in IgAN[3, 4]. Our previous study found that MA lesion was an independent risk factor for IgAN progression[5]. Besides, we also found that MA lesion could occur in normotensive IgAN patients with near-normal renal histology. Thus, MA lesion in

IgAN might not be the result of hypertension alone, the mechanism by which MA lesion occurred in IgAN need to be investigated.

Endothelial cell injury is regarded as the most important cause of MA. In our previous study, we have found that soluble fms-like tyrosine kinase-1 (sFlt-1) levels were significantly lower in IgAN patients than in healthy volunteers. Plasma sFlt-1 levels in IgAN patients correlated with proteinuria, hypertension and von Willebrand factor (vWF) levels[6]. We also found that increased plasma soluble vascular cell adhesion molecule 1 (sVCAM1) was associated with severe clinical and pathological manifestations in IgAN[7]. Heparin-binding EGF-like growth factor (HB-EGF) belongs to epidermal growth factor family, and the HB-EGF/EGFR pathway plays an important role in vascular endothelial cell, which is closely related to tumor growth, coronary artery disease, metabolic disease, and eclampsia[8, 9, 10, 11]. HB-EGF-mediated EGFR signaling is the important pathological mechanism in the renal disease[12]. HB-EGF is critical in the vascular lesions of diabetic nephropathy and hypertensive nephropathy. HB-EGF/EGFR mediated the Ang II/AT1R transactivation in renal tissue damage during hypertension. Our previous unpublished data showed that the HB-EGF level in IgAN patients was significant higher than health control. All these results indicate vascular endothelial injury plays an important role in the progressive of IgAN. However, the causes of endothelial injury are not clear.

The pathophysiologic mechanism of IgAN is very complicated. Recent studies have confirmed the autoimmune nature of IgAN. Aberrant O-glycosylation of the hinge region of circulatory polymeric IgA1 is thought to be the initial pathogenic factor of IgAN. Galactose-deficient IgA1 (Gd-IgA1) lacks galactose on some O-glycans, thus exposing N-acetylgalactosamine. Elevated Gd-IgA1 elicits an autoimmune response, resulting in the generation of anti-glycan antibodies that recognize N-acetylgalactosamine epitopes on Gd-IgA1. The elevation of both Gd-IgA1 and anti-glycan antibodies leads to the formation of a circulating IgA1 complex (cIgA1), which deposits in the glomerular mesangium and induces renal injury [2, 3, 13, 14, 15]. Studies have indicated that cIgA1 deposition in the glomerular mesangium stimulates mesangial cells and induces the secretion of cytokines, chemokines, and extracellular matrix proteins and that the resulting inflammation and fibrosis cause downstream podocyte and tubulointerstitial injury. Recently, Lai hypothesized that there was a crosstalk between glomerular cells and podocytes as well as the immune system and mucosa–bone marrow exists in IgAN, and this crosstalk may be modulated by various environmentally and genetically determined factors as well as the regulation of the complement pathway[15, 16]. However, it is unclear whether cIgA1 can mediate vascular endothelial cell injury. In our previous study, we found that cIgA1 from IgAN patients (IgAN-cIgA1) showed a tendency to increase the sVCAM1 expression in the culture supernatant of human umbilical vein endothelial cells (HUVECs). However, these levels were not different from the levels produced by cIgA1 from healthy controls (HC-cIgA1)[7]. Therefore, IgAN-cIgA1 cannot induce endothelial cell damage directly.

We hypothesize that the mesangial cell–renal vascular endothelial cell crosstalk may be a possible mechanism involved in renal vascular endothelial injury. In the present study, we generate an IgAN endothelial cell injury model *in vitro* and further investigate the mesangial cell–renal vascular endothelial cell crosstalk.

Results

IgAN patients had higher level of vWF, sVCAM-1 and HB-EGF in plasma

59 IgAN patients were involved in the *in vivo* study. Of them, 34 were males and 25 were females. The age at renal biopsy was 42.98 ± 14.36 years. 16 (27.1%) patients had a history of macroscopic hematuria, 22(37.3%) had hypertension, and 25(42.5%) with moderate and severe proteinuria (> 1 g/24 h). Mean 24 h urinary protein excretion (UPE) were 1450.28 ± 1502.79 mg/day. Mean eGFR were 77.89 ± 35.95 ml/min. IgAN patients had higher levels of vWF, sVCAM-1 and HB-EGF than that in healthy controls(912.57 ± 990.96mIU/ml Vs 79.56 ± 63.13mIU/ml, p = 0.001; 550.81 ± 276.00 ng/ml Vs 363.26 ± 86.38 ng/ml, p = 0.005; 24.31 ± 6.74 pg/ml Vs 18.89 ± 7.04 pg/ml, p = 0.004)(Fig. 1). These results indicated that IgAN patients presented with sever endothelial cell injury than healthy control.

IgAN-clgA1 induces the expression of IL-6 and CXCL1 in HMCs

Purified clgA1, isolated from 12 IgAN patients and 10 healthy controls were used to culture HMCs separately. The concentrations of IL-6 and CXCL1 were significantly higher in the conditioned IgAN-HMCM than in the conditioned HC-HMCM (80.92 ± 22.06 pg/ml vs 64.13 ± 13.63 pg/ml, p = 0.049 and 579.89 ± 106.18 pg/ml vs 475.27 ± 120.20 pg/ml, p = 0.04, respectively) (Fig. 2). These results indicated the successful establishment of an *in vitro* activate HMCs model of IgAN.

Cell injury model of HUVECs cultured with IgAN-HMCM

We found that HUVEC growth was more severely impaired in IgAN-HMCM than in HC-HMCM by inverted microscopy (10X) (Fig. 3). Cell proliferation assay showed that HUVEC proliferation was reduced in the IgAN-HMCM group by comparing with the HC-HMCM group (1.41 ± 0.17 vs 1.60 ± 0.23, p = 0.046)(Fig. 4).

The concentrations of vWF, sVCAM1 and HB-EGF in the culture supernatant of HUVECs were measured by ELISA. The levels of vWF, sVCAM1 and HB-EGF were significantly higher in the IgAN-HMCM group than in the HC-HMCM group (20.52 ± 3.22 pg/ml vs 17.42 ± 3.42 pg/ml, p = 0.04; 0.95 ± 0.14 ng/ml vs 0.79 ± 0.14 ng/ml, p = 0.01; 25.35 ± 6.46 pg/ml vs 19.00 ± 3.26 pg/ml, p = 0.01, respectively) (Fig. 5). These results indicated that IgAN-clgA1 could induce endothelial cell injury through mesangial cell activation.

In the present study, we used Panax notoginseng saponins(PNS) as an anti-inflammation intervention factor to the endothelial cell injury *in vitro* cell model. After HUVECs were cultured in PNS and IgAN-HMCM, the cell proliferation assay showed that PNS could promote cell growth in IgA nephropathy(1.65 ± 0.21 vs 1.41 ± 0.17, P = 0.007) (Fig. 3 and Fig. 4). The concentration of vWF, sVCAM1 and HB-EGF in the culture supernatant of these HUVECs was significantly lower than that in the supernatant of HUVECs cultured in IgAN-HMCM alone (16.95 ± 3.30 pg/ml vs 20.52 ± 3.22 pg/ml, p = 0.01; 0.78 ± 0.14 ng/ml vs

0.95 ± 0.14 ng/ml, p = 0.006; 19.90 ± 5.62 pg/ml vs 25.35 ± 6.47 pg/ml, p = 0.038, respectively) (Fig. 6). These results indicated that PNS might have protective effect to endothelial cell injury from the inflammatory cytokines of active mesangial cell.

Discussion

In the present study, we made a cIgA1- HMCM- HUVECs *in vitro* endothelial injury model, in order to investigate whether the mesangial cell could induce endothelial cell injury by the circulation IgA1 complex. We found that the endothelial injury markers including vWF, sVCAM1 and HB-EGF in the IgAN-HMCM group were significantly higher than that in the HC-HMCM group. PNS, which are anti-inflammatory bioactive components of the Chinese medicinal herb Panax notoginseng, could protect HUVECs from being injured by the activated supernatants of HMCs.

The pathology of IgAN is characterized by deposition (or possibly in situ formation) of pathogenic polymeric IgA1 immune complexes in the glomerular mesangium. A ‘multi-hit’ hypothesis has been proposed to explain the pathogenesis of IgAN. In our previous study, we found that IgAN-cIgA1 could not induce endothelial cell injury directly. However, studies had confirmed that cIgA1 deposition could stimulate mesangial cell activation and induce the secretion of cytokines, chemokines, and extracellular matrix proteins, resulting in inflammation and fibrosis, which cause downstream podocyte and tubulointerstitial injury [13, 14, 15]. So in the present study we set a cIgA1-HMCs-HUVECs *in vitro* endothelial injury model to investigate indirect injury mechanism of cIgA1 to vascular endothelial cell in IgAN. Inflammatory cytokines and chemokines secreted by active mesangial cells include transforming growth factor-beta 1 (TGF-β1), IL-6, IL-8, monocyte chemoattractant protein-1, and CXCL1. IL-6 is the main inflammatory factor and the main molecular marker of IgAN progression. CXCL1 is a member of the CXC chemokine family, which has powerful neutrophil chemoattractant activity during inflammation by signaling through the C-X-C motif chemokine receptor2[17]. IgAN-cIgA1 could induce the upregulation of CXCL1 in mesangial cells. In addition, further vitro experiments proved that podocyte injury was induced by mesangial-derived CXCL1 [16]. Urinary CXCL1 is a new predictor of non-invasiveness in IgAN progression [18]. Further CXCL1 elicits its effects during angiogenesis, wound healing and tumorigenesis. CXCL1 induces angiogenesis through CXCR2, extracellular signal–regulated kinases 1/2 and epidermal growth factor pathways in human endothelial cells [19]. Therefore, the inflammatory cytokines and chemokines secreted by active mesangial cells support the mesangial–endothelial cell crosstalk in IgAN. CXCL1 might induce vascular endothelial cell lesions of IgAN. In the present study, cIgA1 considerably increased the levels of IL-6 and CXCL1 in mesangial cells, with a more significant increase found in the IgAN group, which was consistent with previous studies. This result indicated that a successful *in vitro* cell model of IgAN was generated.

vWF, sVCAM1 and HB-EGF are currently used as serological biomarkers of endothelial cell damage. Increased plasma sVCAM1 has previously been association with severe clinical and pathological manifestations in IgAN, which might be partly due to the effect of IgA1 on endothelial cells [7]. In the present study, we found that vWF, sVCAM1 and HB-EGF levels in the IgAN-HMCM group were significantly

higher than those in the HC-HMCM group *in vitro* study as well as *in vivo*, which indicated that IgAN-cIgA1 might be induce endothelial cell injury through mesangial cell activation. To date, few studies have examined vascular lesions in IgAN, and the mechanism by which these lesions develop is not clear. Moreover, there are very few treatments for renal vascular lesions in IgAN. Our study indicated that mesangial–endothelial cell crosstalk is induced by activated mesangial cells.

Panax notoginseng has been widely used as traditional Chinese medicine for a long history. Saponins are one of the main effective components of panax notoginseng[20, 21]. PNS can inhibits adhesion events by the NF-E2-related factor 2 (Nrf2) - p38 - sVCAM1 pathway. PNS have the ability to attenuate oxygen-glucose deprivation/reoxygenation-induced injury by regulating the expression of inflammatory factors through miR-155. PNS protect cerebral microvascular endothelial cells via activation of PI3K/Akt/Nrf2 antioxidant signaling pathway[22, 23, 24]. In the present study, we chose PNS as an anti-inflammation intervention factor. As a result, PNS significantly promoted vascular endothelial cell proliferation and improved vascular endothelial injury. These results further indicated that the endothelial cell injury in IgAN might be the result of inflammation cytokines secreted by cIgA1-induced mesangial cell activation.

There are some limitations in the present study. First, other cytokines on the secretion of factors such as angiotensin II by mesangial cells that could induce endothelial cell injury were not tested in the present study. Second, we did not use the direct inhibitor of CXCL1 or IL-6 in the HUVEC injury model; thus, we cannot conclude that cIgA1 induces HUVEC injury by upregulation of CXCL1 or IL-6. Third, which pathway the protective mechanism of PNS in vascular endothelial cells injury was not investigated.

Conclusions

The cIgA1 could activate mesangial cells and, in turn, induce endothelial cell injury *in vitro*, which might occur through an inflammatory mechanism.

Materials And Methods

Detection of plasma vWF, sVCAM-1 and HB-EGF in IgAN patients

59 IgAN patients and 19 healthy controls were erolled between Jan.2018 and Jan.2019 from Renal Department, Tongde Hospital of Zhejiang Province. IgAN patients were diagnosed by standard examination of the renal biopsy specimen by light microscopy and immunofluorescence, and none of these patients had clinical or serological evidence of Henoch-Schönlein purpura, systemic lupus erythematosus or chronic hepatic diseases. Plasma of these patients and health controls were collected to test the level of markers of endothelial cell injury *in vivo*. In the morning, 3 ml blood (stored in EDTA) from 59 IgAN patients and 19 healthy controls were collected, and stored at − 80 °C. Plasma vWF, sVCAM-1 and HB-EGF levels were determined using commercial ELISA kits according to the manufacturer's specifications (R&D Systems, Minneapolis, MN, USA). At the same time clinical manifestations of the 59

IgAN patients including serum IgA level, serum creatinine level, 24 hours urine protein, history of hypertension, history of gross hematuria and glomerular filtration rate (GFR) were collected from the clinical record. The Study protocol was reviewed and approved by the Ethics Committee of Tongde Hospital of Zhejiang Province. Written informed consent was provided by all participants.

Patients and controls in the *in vitro* study

A total of 12 primary IgAN patients who were diagnosed by renal biopsy in the Renal Division of Peking University First Hospital between Jan. 2016 and Apr. 2016 were examined in this study. Patients were diagnosed by standard examination of the renal biopsy specimen by light microscopy and immunofluorescence. None of the cases had been treated with steroids and/or immunosuppressants, and none of these patients had clinical or serological evidence of Henoch-Schönlein purpura, systemic lupus erythematosus or chronic hepatic diseases. Healthy controls were confirmed as 'healthy' by physical examination with no microscopic hematuria or proteinuria and were comparable to IgAN patients in terms of age and geographical origin. Plasma was collected from IgAN patients and controls for cIgA1 purification and then to build an *in vitro* endothelial cell injury model. The Study protocol was reviewed and approved by the Ethics Committee of Peking University First Hospital. Written informed consent was provided by all participants.

Isolation of circulating IgA1 complexes

In the morning, 10 ml of blood (stored in EDTA) was collected from each enrolled subject. Then, plasma was isolated by centrifugation at 4000 rpm for 15 minutes at 4℃, and samples were immediately frozen at -80 °C until cIgA1 purification. The method of purifying cIgA was established by our lab, and details of the method have been previously described [16]. Briefly, the plasma was diluted 1:2 with 0.01 M phosphate buffered saline (PBS, pH 7.4) and was filtered through a 0.2 μm filter. To purify IgA1, the filtered plasma was first applied to an agarose-bound jacalin affinity chromatography column (Pierce Chemical Company, Rockford, IL, USA), which was equilibrated beforehand with 0.01 M PBS. Then, IgA1 was eluted with 0.1 M melibiose (Sigma, St. Louis, MO, USA) in 0.01 M PBS. The eluted IgA1 was collected and concentrated by 4000 rpm pressure ultrafiltration at 4℃ using a 30KD Vivaspin (Sartorius Stedim Biotech, Sartorius, Göttingen, Germany) to obtain the purified IgA1. Next, the purified IgA1 was applied to a Sephacryl S-300 gel filtration chromatography column (GE Healthcare Life Sciences, Uppsala, Sweden), which was equilibrated beforehand with washing buffer. Three peaks were observed at 280 nm, which represented circulating IgA1 complex (cIgA1), monomeric IgA1 and other small proteins. These fractions were pooled separately and concentrated by pressure ultrafiltration using a 30KD Vivaspin at 4℃. Purified cIgA1 was confirmed by both Western blot analysis and ELISA, and purified samples were stored at -80 °C until used for further experimentation. For experiments, cIgA concentration was measured using a BCA Protein Assay Kit (R&D Systems, Minneapolis, MN, USA).

Culturing primary HMCs with circulating IgA1 complex

Primary HMCs, cell culture media, mesangial cell growth supplement (MsCGS), fetal bovine serum (FBS), penicillin G and streptomycin were purchased from ScienCellTM Corporation (ScienCellTM, Carlsbad, CA,

USA). As previously described [16], HMCs were cultured in human mesangial cell medium (HMCM) supplemented with MsCGS, 5% FBS, penicillin G (100 U/ml) and streptomycin (100 U/ml) at 37 °C in a humidified 5% CO_2 incubator according to the manufacturer's specifications. Fourth-generation cells were selected for the experiment and were seeded into 6-well plates. After cell growth was arrested for 24 hours in HMCM without FBS and MsCGS, HMCs were incubated with 100 μg/ml IgAN-cIgA1 or HC-cIgA1 for 48 hours. After centrifugation, the cell culture supernatants were collected and stored at -80 °C until their use in subsequent experiments. CXCL1 and IL-6 levels are detected in the mesangial-derived supernatant by standard sandwich ELISA assays kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's specifications. Concentration of CXCL1 and IL-6 were calculated according to the standard curve in Elisa kit instructions.

Culturing HUVECs with conditioned medium from HMCs

Primary HUVECs, cell culture media, supplements, FBS, penicillin G and streptomycin were purchased from ScienCellTM Corporation (ScienCellTM, Carlsbad, CA, USA). As previously described [7], cells were cultured in Endothelial Cell Medium supplemented with Endothelial Cell Growth Supplement, 5% FBS, penicillin G (100 U/ml) and streptomycin (100 U/ml) at 37 °C in a humidified 5% CO_2 incubator, according to the manufacturer's specifications. HUVECs were seeded into 6-well plates pre-coated with 1 μg/cm^2 poly-L-lysine. Third-generation cells were selected for the experiment. After 12 hours of starvation, HUVECs were separately incubated with conditioned medium from IgAN-cIgA1-treated HMCs (IgAN-HMCM, 1:8 dilution), conditioned medium from HC-cIgA1-treated HMCs (HC-HMCM, 1:8 dilution), or conditioned medium from HMCs of control group (Control-HMCM, 1:8 dilution) for 6 hours.

Panax notoginseng saponins (PNS), the main bioactive components of a traditional Chinese medicinal herb Panax notoginseng, are used for promoting blood circulation and removing blood stasis [20, 21, 22]. The main mechanisms of PNS function include inhibiting inflammatory cytokines and improving hypoxia [23, 24]. In the present study, PNS was used in HUVEC culture as a preventive control group. PNS was purchased from the GuangXi WuZhou Pharmaceutical Group Co., Ltd. To observe the effect of PNS on vascular endothelial cells in IgAN, HUVECs were incubated with 200 μg/ml PNS combined with HMCM (1:8 dilution) for 6 hours.

vWF, sVCAM1 and HB-EGF levels were detected in the culture supernatants of HUVECs by ELISA. A standard sandwich ELISA was performed using an ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's specifications. Concentration of vWF, sVCAM1 and HB-EGF were calculated according to the standard curve in Elisa kit instructions.

HUVECs Cell Proliferation was assayed by Quick Cell Proliferation Assay Kit (Promega, USA) according to the manufacturer's specifications.

Statistical analysis

For continuous variables, the independent-samples t-test or one-way ANOVA was used if the data were normally distributed, and if not, a Mann-Whitney U test was performed. Data with a normal distribution

were expressed as the mean ± standard deviation, while other data were expressed as the median (5th percentile and 95th percentile). All P values were two-tailed, and values < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS software 23 (IBM).

Abbreviations

TMA
thrombotic microangiopathy, IgAN = IgA nephropathy, HC = health controls, Gd-IgA1 = galactose-deficient IgA1, cIgA1 = circulating IgA1 complex, IgAN-cIgA1 = cIgA1 from IgAN patients, HC-cIgA1 = cIgA1 from healthy controls, HMCs = human mesangial cells, HMCM = human mesangial cell medium, IgA-HMCM = IgA-HMC medium, IgAN-HMCM = IgAN-cIgA1-treated HMCM, HC-HMCM = HC-cIgA1-treated HMCM, MsCGS = mesangial cell growth supplement, FBS = fetal bovine serum, HUVECs = Human umbilical vein endothelial cells, PNS = Panax notoginseng saponins, IL-6 = interleukin-6, CXCL1 = chemokine (C-X-C motif) ligand 1, vWF = von Willebrand factor, sVCAM1 = soluble vascular cell adhesion molecule 1, HB-EGF = Heparin-binding EGF-like growth factor, VEGF = vascular endothelial growth factor, sFlt-1 = soluble fms-like tyrosine kinase-1, TGF-β1 = transforming growth factor-beta1.

Declarations

Ethics approval and consent to participate

Ethical approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of Tongde Hospital of Zhejiang Province and Peking University First Hospital. consent to participate: “Informed consent was obtained from all individual participants included in the study.”

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

YL, LZ and SS conceived and designed the analysis. JL performed the histological examination of the kidney. LZ, HD and XY collected the data. MH performed the cell experiments, analysis and wrote the paper. All authors read and approved the manuscrip.

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Figures

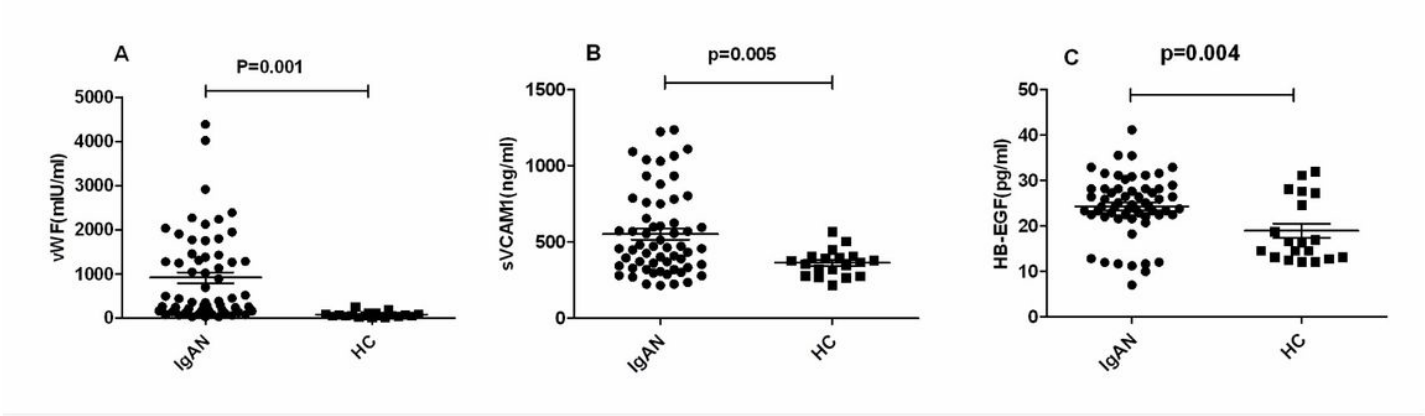

Figure 1

Plasma levels of vWF, sVCAM-1 and HB-EGF in the IgAN patients. Note: a The plasma level of vWF was detected by enzyme-linked immunosorbent assay (ELISA). b The plasma level of sVCAM-1 was detected by ELISA. c The plasma level of HB-EGF was detected by ELISA. Plasma levels of vWF, sVCAM-1 and HB-EGF were significant higher in the IgAN patients than in health control(P<0.001, P<0.001, P<0.001). IgAN, IgA nephropathy group; HC, health controls group.

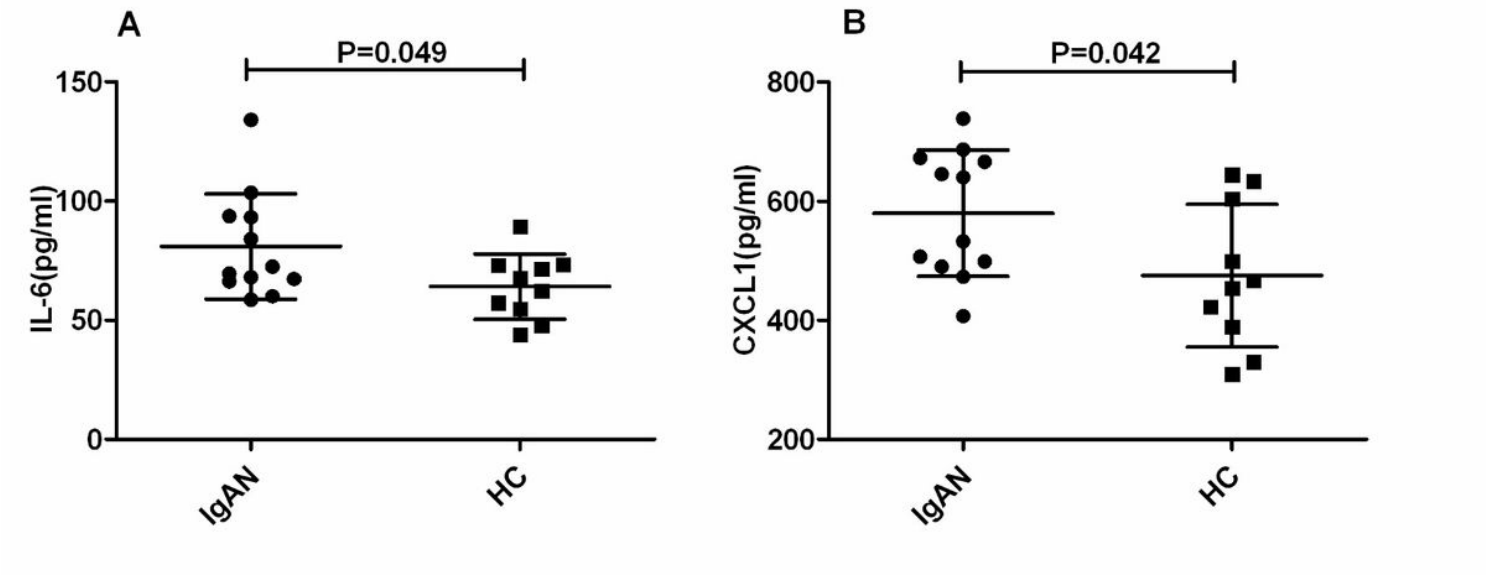

Figure 2

Secretion of IL-6 and CXCL1 by HMCs treated with cIgA1 isolated form IgAN. Note: a The level of IL-6 in the mesangial-derived supernatant was detected by enzyme-linked immunosorbent assay (ELISA). b The level of CXCL1 in the mesangial-derived supernatantwas was detected by ELISA. Secretion of IL-6 and

CXCL1 by HMCs treated with cIgA1 isolated form IgAN were significant higher than that in health control($P<0.05$, $P<0.05$). IgAN, the mesangial cells supernatant derived by cIgA1 from IgAN patients group; HC, the mesangial cells supernatant derived by cIgA1 from health controls group.

Figure 3

The growth of HUVECs observed by inverted microscopy. Note: a The growth of HUVECs was in IgAN-HMCM group(10X). b The growth of HUVECs was in HC-HMCM group(10X). c The growth of HUVECs was

in PNS+IgAN-HMCM group(10X). The growth of HUVECs in IgAN-HMCM group were more severely impaired than that in HC-HMCM groups observed by inverted microscopy. IgAN-HMCM, HUVECs cultured with IgAN-cIgA1-treated human mesangial cell medium group; HC-HMCM, HUVECs cultured with HC-cIgA1-treated human mesangial cell medium group; PNS+IgAN-HMCM group, HUVECs cultured with PNS and IgAN-cIgA1-treated human mesangial cell medium group; PNS, Panax notoginseng saponins.

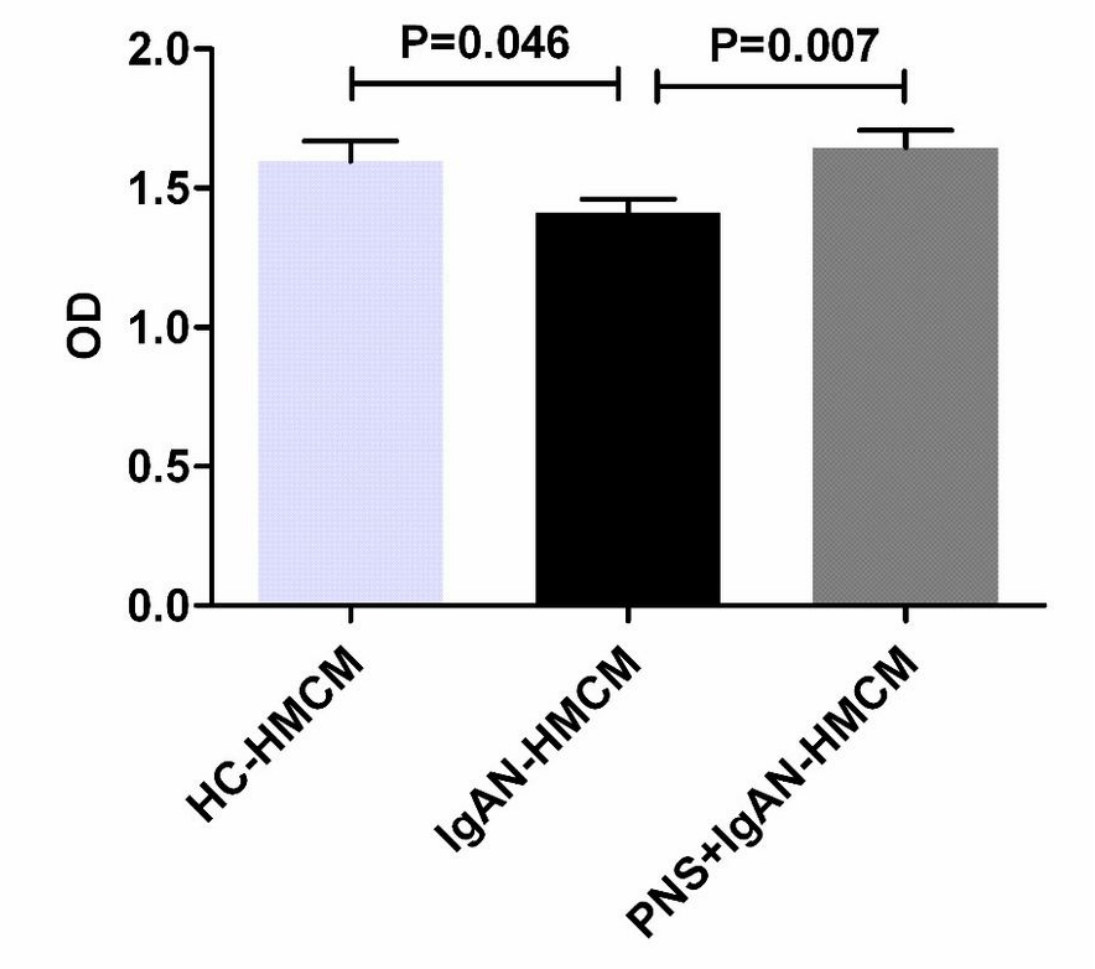

Figure 4

HUVECs Cell Proliferation was assayed by Quick Cell Proliferation Assay. Note: HUVEC proliferation is significantly reduced in the IgAN-HMCM group compared with the HC-HMCM group($P<0.05$). After HUVECs were cultured in PNS and IgAN-HMCM, the cell proliferation assay showed that PNS could promote cell growth in IgA nephropathy($P<0.001$). IgAN-HMCM, HUVECs cultured with IgAN-cIgA1-treated human mesangial cell medium group; HC-HMCM, HUVECs cultured with HC-cIgA1-treated human mesangial cell medium group; PNS, Panax notoginseng saponins; PNS+IgAN-HMCM, HUVECs cultured with PNS and IgAN-HMCM group.

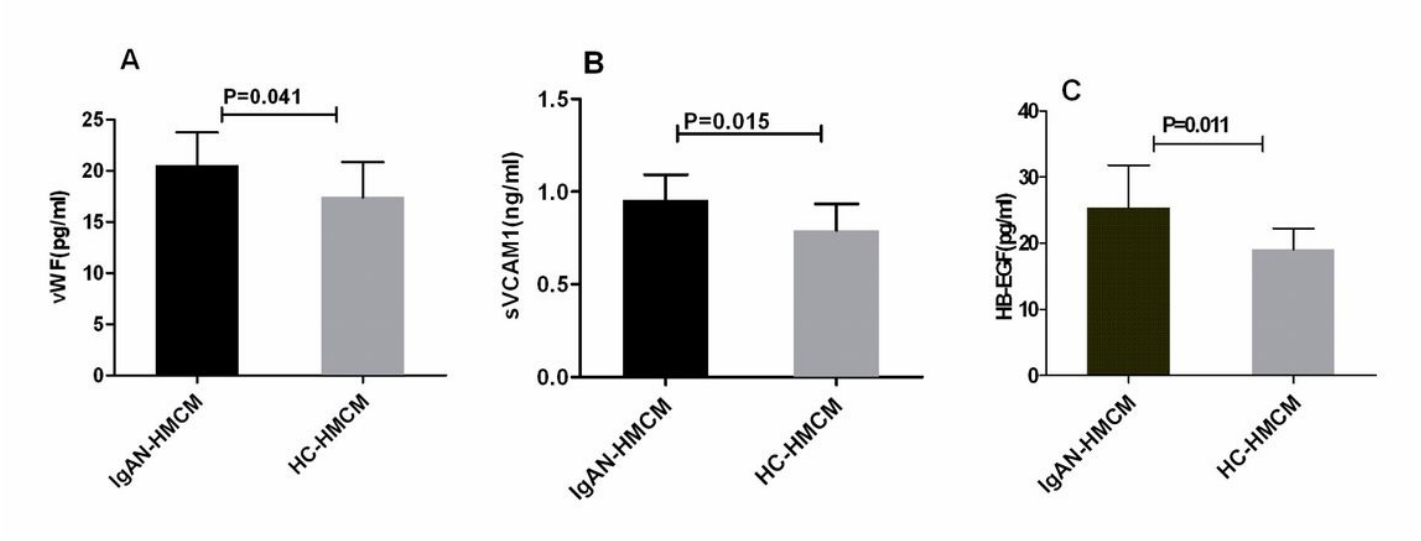

Figure 5

Upregulation of vWF, sVCAM1 and HB-EGF expression in HUVECs cultured with conditioned IgAN-HMCM. Note: a The expression of vWF in HUVECs cultured with HMCM was detected by enzyme-linked immunosorbent assay (ELISA). b The expression of sVCAM1 in HUVECs cultured with HMCM was detected by ELISA. c The expression of HB-EGF in HUVECs cultured with HMCM was detected by ELISA. The expression of vWF, sVCAM1 and HB-EGF in HUVECs cultured with conditioned IgAN-HMCM were significantly higher than that with conditioned HC-HMCM($P<0.05$, $P<0.05$, $P<0.05$). IgAN-HMCM, HUVECs cultured with IgAN-cIgA1-treated human mesangial cell medium group; HC-HMCM, HUVECs cultured with HC-cIgA1-treated human mesangial cell medium group.

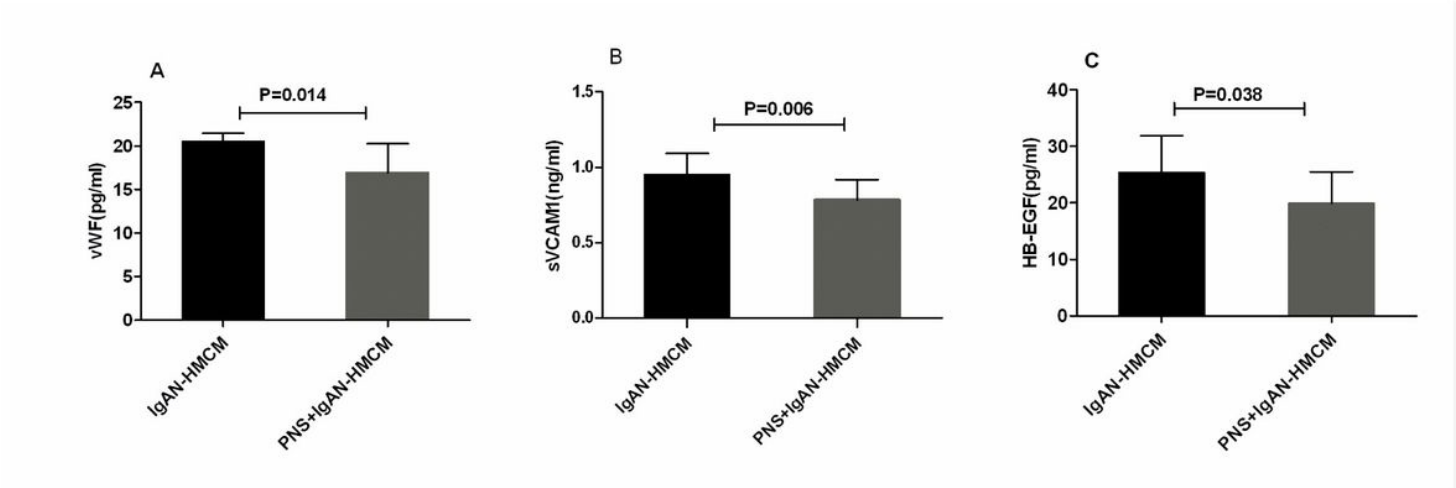

Figure 6

The protective function of PNS on vascular endothelial cell injury in vitro. Note: a The expression of vWF in HUVECs cultured with HMCM was detected by enzyme-linked immunosorbent assay (ELISA). b The expression of sVCAM1 in HUVECs cultured with HMCM was detected by ELISA. c The expression of HB-EGF in HUVECs cultured with HMCM was detected by ELISA.The expression of vWF, sVCAM1 and HB-EGF was reduced significantly after HUVECs were cultured in conditioned medium together with PNS($P<0.05$, $P<0.001$, $P<0.05$). IgAN-HMCM, HUVECs cultured with IgAN-clgA1-treated human mesangial cell medium group; PNS+IgAN-HMCM, HUVECs cultured with PNS and IgAN-clgA1-treated human mesangial cell medium group.