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NEPHROLOGY - ORIGINAL PAPER

Mycophenolic acid reverses IgA_1 aberrant glycosylation through up-regulating Cosmc expression in IgA nephropathy

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Abstract
Objective Impaired core I β3-Gal-T-specific molecular chaperone (Cosmc) expression-caused IgA_1 aberrant O-glycosylation is one of the main pathogeneses of IgA nephropathy (IgAN).This study tried to elucidate whether mycophenolic acid (MPA) could up-regulate Cosmc expression of peripheral lymphocytes in IgAN patients and reverse the dys-O-glycosylation.
Method Peripheral lymphocytes of eighteen IgAN patients and twelve normal controls were isolated and cultured for 3–7 days with or without lipopolysaccharide (LPS) and MPA. Cosmc mRNA and protein expression levels were measured by real-time RT-PCR and western blot. IgA_1 and O-glycosylation level were determined by enzyme-linked immunosorbent assay (ELISA) and VV lectin-binding test. Correlation analysis was performed between Cosmc expression levels and IgA_1 O-glycosylation level.
Results Cosmc mRNA expression and IgA_1 O-glycosylation level in IgAN patients were significantly lower than normal controls. Treatment of LPS could obviously inhibit the Cosmc expression and increase the IgA_1 secretion in peripheral lymphocytes of IgAN patients, which resulted in a significantly increase in IgA_1 aberrant glycosylation level. Addition of MPA could significantly increase the Cosmc expression level along with a decrease in IgA_1 secretion, leading to a reverse of aberrant glycosylation. A significant positive correlation between the Cosmc expression and IgA_1 O-glycosylation level was noticed.
Conclusion MPA can up-regulate the Cosmc expression and reverse the IgA_1 aberrant O-glycosylation level in peripheral lymphocytes of IgAN patients, which might be the underlying mechanism of mycophenolate mofetil (MMF) therapy used in treating IgAN.

Keywords IgA nephropathy · Glycosylation · Cosmc · Mycophenolic acid

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Introduction

IgA nephropathy (IgAN) is one of the most common glomerulonephritis all over the world, accounting for >50 % of biopsy-proven primary glomerulonephritis

in Asia [1]. Recent investigations indicated that abnormalities of IgA_1 O-glycosylation may be one of the key pathogeneses of IgAN [2, 3]. Decreased Cosmc (core I β3-Gal-T-specific molecular chaperone) mRNA expression level may be the underlying mechanism of IgA_1 dys-glycosylation [4–6]. Mycophenolate mofetil (MMF) has been successfully used in treating IgAN for many years, which gives us an inspiration that MMF may up-regulate the Cosmc expression and reverse the aberrant glycosylation of IgA_1 molecules in IgAN patients. In the present study, we aimed to elucidate whether recovery of Cosmc expression and dys-glycosylation are the mechanisms of therapeutic effect of MMF in treating IgAN.

Materials and methods

Patients and normal controls

Eighteen biopsy-proven IgAN patients were included in this study after informed consent was obtained. Diagnosis criterion of IgAN was based on generalized glomerular mesangial proliferation with the presence of IgA as the sole or predominant immunoglobulin deposition in renal biopsy. Patients had never received corticosteroids or other immunosuppressive therapy before sample collection. Patients with systemic diseases such as Schonlein–Henoch purpura, systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus, or liver cirrhosis were excluded. Twelve age- and sex-matched healthy volunteers were selected as normal controls after informed consent was obtained. Measurements of blood pressure (BP), urinalysis and serum creatinine were performed to exclude those who had abnormal findings.

Lymphocyte isolation

Peripheral lymphocytes were obtained following a previously reported method [5]. Briefly, 20 ml of venous blood sample was collected in EDTA-anticoagulated tubes. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation using Lymphocyte-H lymphocyte isolation media (Cedarlane Laboratories Limited, Canada). Monocytes were depleted by wall-sticking method for 2 h. Cell morphology was monitored using a phase-contrast microscope, and cell viability was detected by trypan blue dye staining which showed that cell activation >95 %.

Lymphocyte culture and treatment

Lymphocytes were cultured (10^6 cells/ml) with complete RPMI-1640 medium containing 15 % fetal calf serum + L-glutamine 2 mM, HEPES 1 mM, penicillin 100 U/mL and streptomycin 100 mg/mL in 24-well plates at 37 °C. Pokeweed mitogen (PWM, 5.0 μg/mL) was added to aid the proliferation and differentiation. Lymphocytes were divided into six groups including IgAN (A, B and C) and normal controls (a, b and c): groups A and a (Blank): RPMI + PWM; groups B and b (LPS): RPMI + PWM + LPS; and groups C and c (MPA): RPMI + PWM + LPS + MPA. The concentrations of LPS and MPA were 12.5 μg/mL and 5.0 μmol/L, respectively. Cells were cultured in 24-well plates at 37 °C for 3–7 days. Viability of PBMCs was about 85 % as determined by trypan blue. Flow cytometric analysis with two-color staining was used to determine the differentiation of lymphocytes. B cells were defined as CD19+/CD38–, whereas plasma cells were defined as CD19–/CD38+.

IgA_1 ELISA analysis and Vicia villosa lectin-binding assay

IgA_1 concentration of cell culture supernatants was determined by ELISA. As previously described [5, 6], 96-well plates were coated with primary antibody (Southern Biotechnology Associates, USA) overnight at 4 °C. After blocking, samples were added in duplicate and incubated for 1 h at 37 °C with biotinylated secondary antibody (Southern Biotechnology Associates, USA). After another 1-h incubation at 37 °C with peroxidase-avidin D (Vector Laboratories, UK), color was developed using tetramethyl benzidine dilution (TMB) and detected at 450 nm. The concentration of IgA_1 was calculated from the standard curve that was constructed with a serial dilution of IgA_1 standard serum (Nordic Immunological Laboratories, Netherlands). The levels of IgA_1 O-glycosylation were determined by Vicia villosa (VV) lectin-binding assay as previously described [5, 6]. Briefly, 96-well plates were coated with primary antibody and blocked as described above. Samples were added in duplicate and incubated for 1 h at 37 °C. After incubation for 1 h at

37 °C with biotinylated VV lectin (Vector Laboratories, UK), washed again, and peroxidase-avidin D (Vector Laboratories), color was developed and detected as above. As results obtained from different assays run at different times could not be compared directly, we included a same control serum as internal calibrator in each assay. For each sample, the observed optical density (OD) was calibrated according to the OD of calibrator sample, allowing for further comparisons.

Cosmc gene qPCR quantification

Total RNA was extracted from lymphocytes using the RNeasy Mini kit (QIAGEN, USA). Real-time quantitative PCR (RT-PCR) was performed with the Taqman probe technique after reverse transcription. The primers and probes of Cosmc and GAPDH (internal control) are listed in Table 1 (synthesized by Invitrogen, China). The PCR was performed in a Roche Lightcycler (Roche Diagnostics, USA) as previously described [5]. In order to examine the efficiency of RT-PCR, standard curves were established with serial dilutions of sample RNA (500 ng; 10 × dilution). PCR products were purified and sequenced directly (Invitrogen, China). The sequencing result showed that the amplified fragment was in accordance with the GenBank record.

Cosmc protein quantification

Cosmc protein quantification was performed using western blot as previously reported [7]. Protein samples of lymphocytes were separated on 10 % sodium dodecyl sulfate polyacrylamide (SDSXie PAGE) gels and transferred to polyvinylidene fluoride (PVDF) membranes. After being blocked for 1 h, blots were incubated with primary antibodies (1:200; Santa Cruz Biotechnology, USA) overnight at 4 °C. After washing, the blots were incubated with horseradish peroxidase-linked secondary antibodies (1:5000; Santa Cruz Biotechnology, USA) for 1 h. After further washing, the immunoreactivities of antibodies were detected via ECL reagents (GE Healthcare, USA). The measurement of *beta*-actin was applied as an internal calibrator.

Statistical analysis

Paired *t* test analyses and ANOVA test were performed to evaluate the changes in IgA_1 and VV lectin-binding levels. Delta Ct was used in the analysis of Cosmc mRNA qPCR analysis. *p* value of 0.05 was taken as the level of statistical significance. Pfaffl's method, the most accepted method of relative qPCR analysis, was applied during the analysis of real-time PCR results. Pearson correlation analysis was used to evaluate the correlation between Cosmc expression level and IgA_1 VV lectin-binding capacity.

Results

General features of subjects included

The clinical characteristics of the subjects are shown in Table 2. No significant difference was observed in the age, sex and ethnological background of IgAN patients and normal controls included ($p > 0.05$).

Effects of MPA on IgA_1 secretion and glycosylation

In IgAN patients, after the treatment of LPS, IgA_1 concentration in culture medium increased much higher than in normal controls. However, MPA treatment could apparently inhibit IgA_1 secretion of lymphocytes from IgAN patients (Fig. 1; Table 3).

Baseline VV lectin-binding level of IgAN patient was significantly higher than that of normal control, indicating a higher aberrant glycosylation level. After LPS stimulation, the VV lectin-binding level increased

Table 1 Primers and fluorescence probes

	Cosmc	GAPDH
Sense	5-GTAACGGAGTGGTGCGCCAA-3	5-GGGTGTGAACCATGAGAAGT-3
Antisense	5-TTGCACTTCATCCGCGTCTAGA-3	5-CCAAAGTTGTCATGGATGACCT-3
Probe	5-FAM-CGTGCGCGGCTGCGCTTTCCT-TAMRA-3	5-FAM-CTGCACCACCAACTGCTTAGC-TAMRA-3

Table 2 Baseline clinical characters of subjects included

	IgAN ($n = 18$)	Normal controls ($n = 12$)
Age (years)	32.2 ± 6.1*	29.5 ± 4.2*
Males/females	10/8	7/5
Disease duration (months)*	24.2 ± 15.7	
Blood pressure systolic (mmHg)	112.7 ± 21.4*	108.5 ± 15.5*
Blood pressure diastolic (mmHg)	70.5 ± 7.5 *	65.5 ± 6.8*
Serum creatine (μmol/L)	115.4 ± 13.8	98.3 ± 3.2
Urine protein (g/24 h)	3.88 ± 1.27	0.10 ± 0.01

* Expressed as mean ± standard deviation

dramatically ($p < 0.01$, comparison with Blank group). After MPA treatment, VV lectin-binding level decreased. On the 3rd and 5th day, there was no significant difference between LPS and MPA groups in VV lectin-binding levels, but on the 7th day, a significant difference was found ($p < 0.01$). However, no remarkable differences were observed between every group in normal controls (Fig. 2; Table 4).

Effects of MPA on Cosmc gene expression

Standard curves were established to examine the efficiency of real-time PCR. Efficiency and correlation index (R^2) were >0.99. The slope of Cosmc and GAPDH PCR was −3.377 and −3.581, respectively. According to the principle of real-time PCR, the higher the Ct value is the lower the mRNA level presents.

Baseline Cosmc mRNA expression level in peripheral lymphocytes of IgAN patients was significantly lower than that of normal controls. Removing the serum and culturing the lymphocytes with RPMI-1640 medium alone (BLK) could increase the Cosmc gene mRNA expression level remarkably. However, co-culture with LPS obviously inhibited the up-regulation of Cosmc expression. When MPA was added, the Cosmc mRNA expression increased apparently. For normal controls, no significant change was observed in Cosmc mRNA expression after abovementioned treatment (Fig. 3; Table 5).

In order to compare the Cosmc expression levels in a more straightforward way, Pfaffl's method was applied [8]. Baseline Cosmc gene expression level in IgAN patients was only 59 % of normal control. Culturing with RMPI-1640 medium increased the

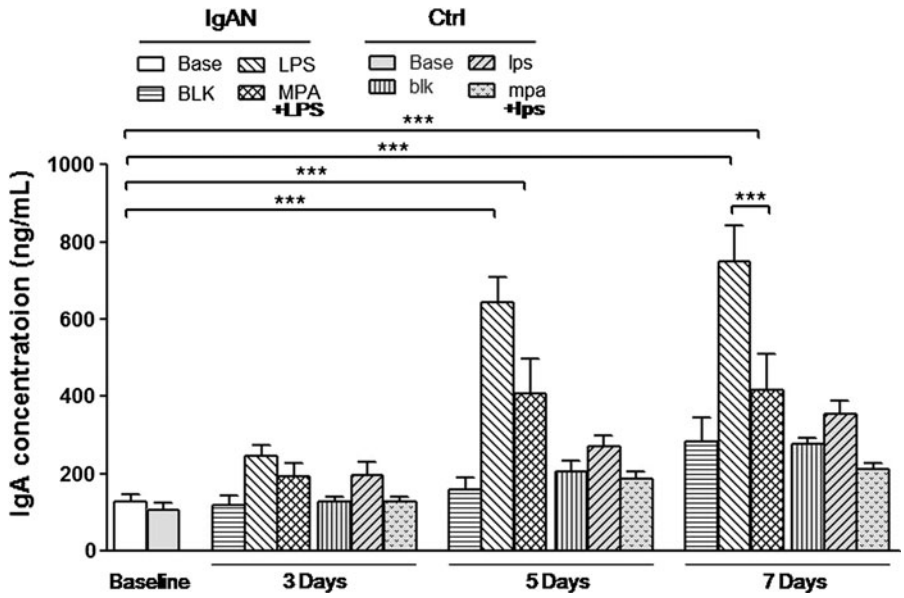

Fig. 1 IgA_1 (ng/mL) concentration in supernatant from IgAN patients' and normal controls' peripheral lymphocytes. After LPS stimulation, lymphocytes from either patients with IgAN or normal controls secreted high concentration of IgA_1, especially from IgAN patients. However, treatment with MPA leads to a decrease in the secretion of IgA_1

Table 3 IgA1 concentration (ng/mL) secreted by cultured lymphocytes in IgAN patients and normal controls

		IgAN ($n = 10$)	Normal controls ($n = 8$)
Baseline		127.4 ± 20.4[a, b, c, d]	107.3 ± 17.9
Days 3	Blank	119.4 ± 22.5	129.4 ± 11.9
	LPS + PWM	247.2 ± 25.2	195.5 ± 35.7
	LPS + PWM + MPA	192.8 ± 34.4	127.1 ± 13.8
Days 5	Blank	157.7 ± 30.8	206.6 ± 26.5[f]
	LPS + PWM	642.0 ± 65.8[a]	270.3 ± 30.0[f]
	LPS + PWM + MPA	407.1 ± 90.6[b]	186.0 ± 18.1
Days 7	Blank	282.9 ± 63.8	276.5 ± 15.3 [g]
	LPS + PWM	749.8 ± 91.0[c, e]	353.9 ± 35.7 [g]
	LPS + PWM + MPA	417.0 ± 91.8[d, e]	211.4 ± 16.1

[a, b, c, d, e, f, g] comparison between 2 groups $p < 0.05$

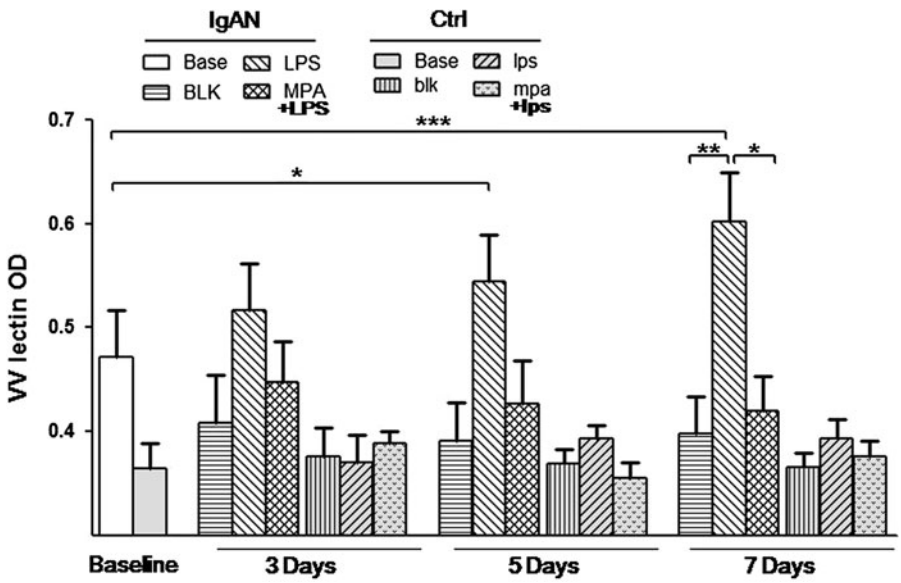

Fig. 2 IgA_1 O-glycosylation assay (OD) in supernatant of IgAN patients and normal controls peripheral lymphocytes. LPS induced significant inhibition of glycosylation in IgAN patients, which could be reversed by MPA, while no significant difference was observed in normal controls

Cosmc mRNA expression level dramatically (205, 200 and 246 % for 3rd, 5th and 7th day, respectively). However, co-culturing with LPS resulted in apparently inhibition (49, 49 and 16 % on 3rd, 5th and 7th day comparing with RMPI-1640 culture). Nevertheless, MPA could reverse the effect of LPS, Cosmc expression increased to 92, 157 and 210 % of baseline level on 3rd, 5th and 7th day, respectively (Fig. 4).

Western blot was applied to measure the Cosmc protein expression levels in each group. Results were in accordance with that of qPCR: after RPMI-1640 culture, the Cosmc protein expression increased, and LPS treatment inhibited the Cosmc protein expression strongly. MPA could significantly reverse the expression of Cosmc protein from 3rd to 7th day (Fig. 5).

Correlation between Cosmc expression and O-glycosylation

The correlation between Cosmc protein level and IgA_1 O-glycosylation level was analyzed (Fig. 6). Pearson correlation analysis indicated that there was a significant positive correlation between the expression of Cosmc protein and IgA_1 O-glycosylation ($r = 0.828$, $p = 0.006$).

Table 4 VV lectin binding of IgA1 in lymphocytes culture supernatant in IgAN patients and normal controls

		IgAN ($n = 18$)	Normal controls ($n = 12$)
Baseline		$0.47 \pm 0.05^{a, b, c}$	0.36 ± 0.02^{a}
Days 3	Blank	0.41 ± 0.05	0.38 ± 0.03
	LPS + PWM	0.52 ± 0.04	0.37 ± 0.02
	LPS + PWM + MPA	0.45 ± 0.04	0.39 ± 0.02
Days 5	Blank	0.39 ± 0.04	0.37 ± 0.01
	LPS + PWM	0.54 ± 0.05^{b}	0.39 ± 0.02
	LPS + PWM + MPA	0.43 ± 0.04	0.36 ± 0.01
Days 7	Blank	0.40 ± 0.04^{d}	0.37 ± 0.01
	LPS + PWM	$0.60 \pm 0.05^{c, d, e}$	0.39 ± 0.02
	LPS + PWM + MPA	0.42 ± 0.03^{e}	0.38 ± 0.02

$^{a, b, c, d, e}$ comparison between 2 groups $p < 0.05$

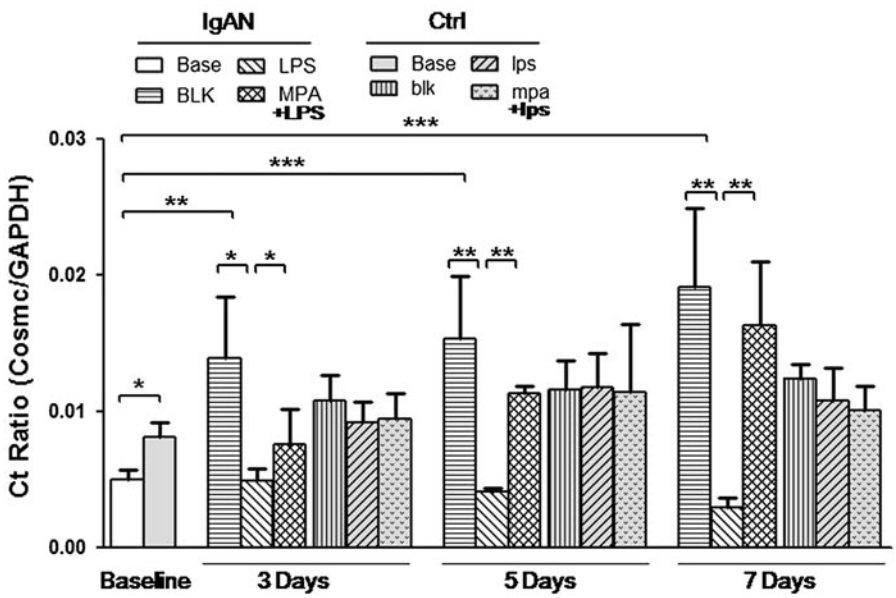

Fig. 3 The expression levels of Cosmc mRNA represented by Delta Ct. LPS stimulation significantly suppressed the Cosmc mRNA expression, but addition of MPA could reverse its expression

Discussion

IgAN is the most common glomerulonephritis in the world, especially in Asian countries. Patients with nephrotic syndrome, renal dysfunction, massive hematuria and advance histopathological lesions may have poor prognosis [9]. The pathogenesis of IgAN is still unclear. Genetic disorders, environment stimulations, immunologic abnormalities and oxidative stress participate in the development of the disease [10]. Abnormality of IgA_1 hinge region O-glycosylation may be one of the causes of IgAN [1–3]. Previous studies indicated that O-linked glycans were significantly decreased in sera, mesangial deposited and tonsil secreted IgA_1 molecules in IgAN patients and the glycosylation aberrance were closely associated with pathologic phenotypes [11]. It is reported that O-glycans are formed under the catalysis of β1,3-galactosyltransferases and its specific functional chaperon (Cosmc) [12]. However, Cosmc gene mRNA expression level was remarkably lower in peripheral B lymphocyte of IgAN patients, which resulted in a lower β1,3 galactosyltransferase synthesis activity and IgA glycosylation level as well as severer clinical

Table 5 Ct value of each group in IgAN patients and normal controls

		IgAN ($n = 18$)	Normal controls ($n = 12$)
Baseline		$0.005 \pm 0.0007^{a, b, c, d}$	0.008 ± 0.0011^{a}
Days 3	Blank	$0.014 \pm 0.0045^{b, e}$	0.011 ± 0.0019
	LPS + PWM	$0.005 \pm 0.0008^{e, f}$	0.009 ± 0.0015
	LPS + PWM + MPA	0.008 ± 0.0026^{f}	0.010 ± 0.0018
Days 5	Blank	$0.015 \pm 0.0045^{c, g}$	0.012 ± 0.0021
	LPS + PWM	$0.004 \pm 0.0003^{g, h}$	0.012 ± 0.0026
	LPS + PWM + MPA	0.011 ± 0.0006^{h}	0.011 ± 0.0050
Days 7	Blank	$0.019 \pm 0.0058^{d, k}$	0.012 ± 0.0011
	LPS + PWM	$0.003 \pm 0.0007^{k, m}$	0.011 ± 0.0024
	LPS + PWM + MPA	0.020 ± 0.0046^{m}	0.010 ± 0.0018

$^{a, b, c, d, e, f, g, h, k, m}$ comparison between 2 groups $p < 0.05$

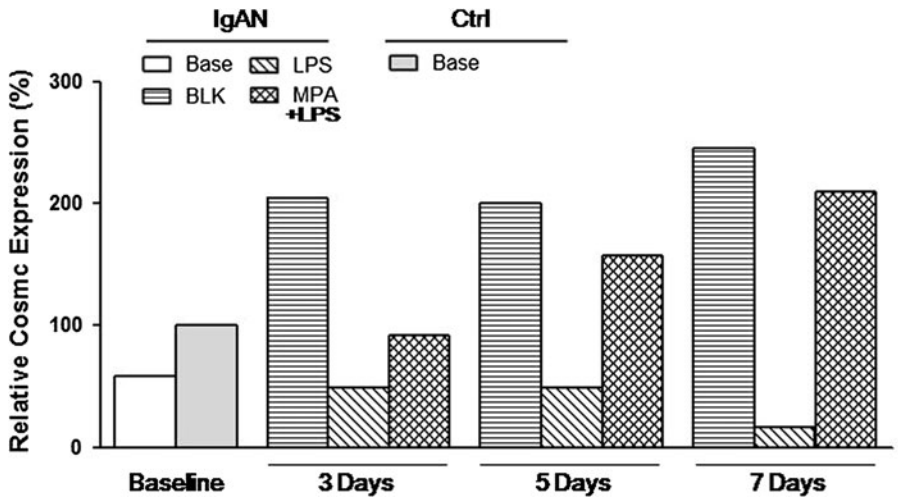

Fig. 4 The relative expression levels of Cosmc mRNA in IgAN patients represented by Pfafffl's analysis

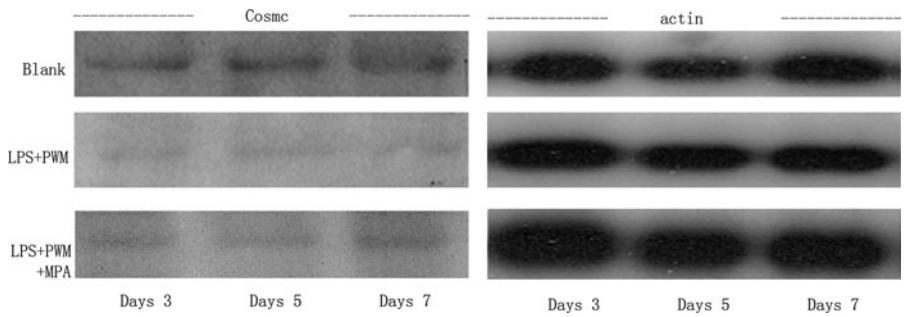

Fig. 5 Western blot analysis for Cosmc protein after cultured in presence or absence of LPS, MPA. Representative western blots of Cosmc proteins for the IgAN patients

manifestations [4–6]. Therefore, reverse of Cosmc gene expression level might be an important treatment target of IgAN.

Previously study indicated that treatment with 5-azacytidine could reactivate the suppressed β1,3GT activity [13] and could up-regulate Cosmc expression and improve IgA_1 O-glycosylation[7], which might help to identify new targets for future treatments of IgAN. However, 5-AZA has not been used in clinical practice, and therefore, finding a clinical available

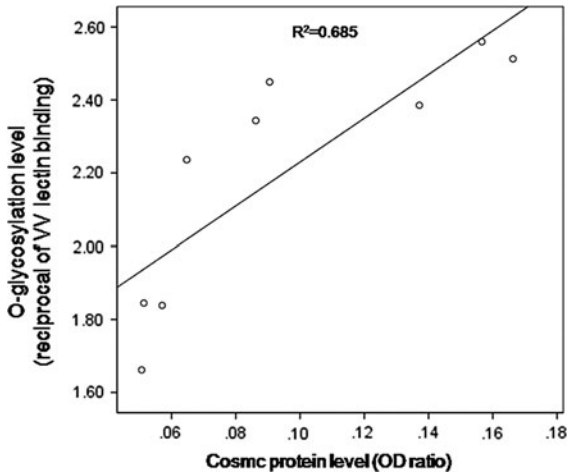

Fig. 6 Correlation between Cosmc expression and O-glycosylation levels of IgA_1. Glycosylation levels are represented in reciprocal of VV lectin-binding level (OD450) and the Cosmc expression shown as the absorbance density of Cosmc proteins (Cosmc/actin ratio)

drug which could regulate the Cosmc expression and IgA_1 glycosylation level may be helpful to the treatment of IgAN.

Mycophenolate mofetil (MMF) can inhibit antibody production by B cells stronger than other immunosuppressants. Several randomized controlled trials (RCTs) have investigated the role of MMF in patients with IgAN. In Asian IgAN subjects, MMF could effectively lower proteinuria level in the short-term and lead to reno-protection effect in the long-term [14, 15]. However, meta-analysis of several RCT studies revealed that MMF appeared to be effective in reducing proteinuria in Chinese but not Caucasoid IgAN subjects [16]. The underlying cause of this difference is yet to know. Regarding that Cosmc is crucial to the IgA_1 glycosylation and pathogenesis of IgAN, we hypothesized that ethnic function and structure variety of Cosmc may account for the variation of MMF effects observed in these studies.

In the current study, we found that peripheral lymphocytes from IgAN patients secreted significantly higher level of IgA_1 compared with normal controls at baseline and under LPS stimulation. VV lectin-binding assay also indicated that aberrant IgA_1 O-glycosylation levels of IgAN patients' peripheral lymphocytes were prominently higher than normal control subjects at baseline and after LPS treatment. Further qPCR and western blot showed that Cosmc gene expression in IgAN patients' lymphocytes were much lower than that of normal controls at baseline and after LPS treatment. These data indicated that in IgAN patients, during the LPS stimulation, more IgA_1 was secreted while Cosmc gene was suppressed, which resulted in disequilibrium between the glycosylation demand and supply. In the previous study, flow cytometry analysis and MTT test indicated that that LPS and PWM can evoke the cell proliferation in a synergistic effect along with an increase in plasma cells accompanied by the enhanced concentration of IgA_1 with increasing time, implying that IgA_1 was secreted by the increased number of plasma cells in each group. Moreover, the proliferative and differentiative effects of these drugs are more potent in peripheral lymphocytes of IgAN patients [7]. These data were in accordance with our previous findings that external inhibitors such as LPS could affect the glycosylation levels of IgA_1 through the regulation of Cosmc gene expression, and it was the Cosmc expression abnormalities that responsible for the IgA_1 aberrant O-glycosylation. However, after the addition of MPA, over-secretion of IgA_1 was reversed along with up-regulated Cosmc expression, which resulted in recovery of IgA_1 O-glycosylation level.

Mycophenolate mofetil is a potent immunosuppressant; selective inhibition of lymphocyte proliferation and antibody formation were usually considered as the underlying mechanism of its therapeutic effect. It was reported that MPA could inhibit the proliferative response of human peripheral blood mononuclear cells to phytohemagglutinin, pokeweed mitogen and Staphylococcus protein A Sepharose. Antibody formation by polyclonally activated human B-lymphocytes was almost completely inhibited by MPA [17]. However, studies focused on the effect of MMF on IgA glycosylation were still lacking. It was reported that mean serum IgA concentration and mesangial binding of pIgA decreased significantly after MMF treatment [15]. Considering that dys-O-glycosylation of IgA leads to decreased IgA removal and increased pIgA formation, these results may reflect a reverse of IgA_1 dys-O-glycosylation. Hence, in the current study, we tried to clarify the effect of MMF on Cosmc gene expression and IgA_1 glycosylation level.

The present study showed that treatment with MPA could apparently inhibit the secretion of IgA_1 induced by LPS stimulation. Meanwhile, the expression levels of Cosmc mRNA increased dramatically after MPA treatment, along with the recovery of IgA_1

O-glycosylation. These results indicated that, after treatment with MPA, the equilibrium between glycosylation demand and capacity was reestablished, resulting in normal IgA_1 O-glycosylation. Previous study indicated that deficiency of tonsillar and peripheral lymphocytes Cosmc gene expression was prominent in Aisan IgAN patients, while not in Caucasoid IgAN patients [18]. Judging from the results obtained in this study, we speculated that Asian IgAN patients treated with MPA could restore the Cosmc gene expression and reverse the IgA_1 dys-glycosylation which led to proteinuria decreasing and renoprotection effects observed in clinical practice. However, as no Cosmc expression disorder was noticed in Caucasoid IgAN patients, MPA treatment may therefore not effective.

The most important limitation of this study is that the effect of MMF on the IgA_1 glycosylation in patients was not measured. Although in vitro studies can shed light on the therapeutic mechanism of MMF in IgAN patients, further studies are needed to clarify this directly. However, this is very difficult, because other drugs such as glucosteroids and ARB/ACEI are always prescribed along with MMF in treating IgAN patients. Therefore, the changes in patients IgA1 glycosylation level may also be resulted from the use of other drugs. A random controlled trial comparing MMF and other drugs might be helpful to elucidate it.

Conclusion

Cosmc expression was closely associated with IgA_1 O-glycosylation in IgAN. MPA can up-regulate the Cosmc gene expression and reverse the IgA_1 aberrant O-glycosylation in peripheral lymphocytes of IgAN patients under LPS treatment. It may be an underlying mechanism of the therapeutic effect of MMF in the treatment of IgA nephropathy.

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References

1. Barratt J, Feehally J (2005) IgA nephropathy. J Am Soc Nephrol 16(7):2088–2097
2. Giannakakis K, Feriozzi S, Perez M et al (2007) Aberrantly glycosylated IgA1 in glomerular immune deposits of IgA nephropathy. J Am Soc Nephrol 18(12):3139–3146
3. Narita I, Gejyo F (2008) Pathogenetic significance of aberrant glycosylation of IgA1 in IgA nephropathy. Clin Exp Nephrol 12(5):332–338
4. Allen AC, Topham PS, Harper SJ et al (1997) Leucocyte beta 1,3 galactosyltransferase activity in IgA nephropathy. Nephrol Dial Transplant 12(4):701–706
5. Qin W, Zhou Q, Yang LC et al (2005) Peripheral B lymphocyte beta1, 3-galactosyltransferase and chaperone expression in immunoglobulin A nephropathy. J Intern Med 258(5):467–477
6. Qin W, Zhong X, Fan JM et al (2008) External suppression causes the low expression of the Cosmc gene in IgA nephropathy. Nephrol Dial Transplant 23(5):1608–1614
7. Xie LS, Qin W, Fan JM et al (2011) The role of C1GALT1C1 in lipopolysaccharide-induced IgA1 aberrant O-glycosylation in IgA nephropathy. Clin Invest Med 33(1):E5–E13
8. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29(9):e45
9. Moriyama T, Nakayama K, Iwasaki C et al (2012) Severity of nephrotic IgA nephropathy according to the Oxford classification. Int Urol Nephrol 44(4):1177–1184
10. Zhu C, Mertens PR (2012) IgA nephropathy and oxidative stress: news on clinically evaluated biomarkers hits the stage. Int Urol Nephrol 44(4):1277–1280
11. Xu LX, Zhao MH (2005) Aberrantly glycosylated serum IgA1 are closely associated with pathologic phenotypes of IgA nephropathy. Kidney Int 68(1):167–172
12. Ju T, Cummings RD (2002) A unique molecular chaperone Cosmc required for activity of the mammalian core 1 beta 3-galactosyltransferase. Proc Natl Acad Sci USA 99(26): 16613–16618
13. Felner KM, Dinter A, Cartron JP et al (1998) Repressed beta-1,3-galactosyltransferase in the Tn syndrome. Biochim Biophys Acta 1406(1):115–125
14. Tang SC, Tang AW, Wong SS et al (2010) Long-term study of mycophenolate mofetil treatment in IgA nephropathy. Kidney Int. **77**(6): 543–549
15. Tang S, Leung JC, Chan LY et al (2005) Mycophenolate mofetil alleviates persistent proteinuria in IgA nephropathy. Kidney Int 68(2):802–812
16. Xu G, Tu W, Jiang D et al (2009) Mycophenolate mofetil treatment for IgA nephropathy: a meta-analysis. Am J Nephrol 29(5):362–367
17. Allison AC (2005) Mechanisms of action of mycophenolate mofetil. Lupus 14(Suppl 1):s2–s8
18. Inoue TH, Sugiyama M, Kitagawa et al (2011) Abnormalities of glycogenes in tonsillar lymphocytes in IgA nephropathy. Adv Otorhinolaryngol 72:71–74