Article

Imbalance of Interleukin 18 and Interleukin 18 Binding Protein in Patients with Lupus Nephritis

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To evaluate the balance status of interleukin 18 (IL-18) and interleukin 18 binding protein (IL-18BP) in circulation in patients with lupus nephritis (LN) and primary nephrotic syndrome (PNS), plasma levels as well as mRNA expression in peripheral blood mononuclear cells (PBMCs) of IL-18 and IL-18BP were measured by ELISA and RT-PCR respectively. The ratio of IL-18/IL-18BP was also calculated. Both plasma IL-18 and IL-18BP increased significantly in LN patients while only IL-18BP increased in PNS, which resulted in an elevated ratio of IL-18/IL-18BP in LN but not in PNS patients when compared with normal controls. In contrast, increased level of IL-18 mRNA was only detected in LN but not in PNS group, although IL-18BP mRNA expressions in PBMCs in both groups were higher than that in control. The imbalance of IL-18 and IL-18BP might be involved in the pathogenesis of LN, based on which a therapeutic approach is valuable to be developed for LN. *Cellular & Molecular Immunology*. 2006;3(4): 303-306.

Key Words: IL-18, IL-18 binding protein, lupus nephritis, primary nephrotic syndrome

Introduction

Immune dysregulation, particular cytokine secretion imbalance plays a crucial role in many human kidney diseases. Interleukin 18 (IL-18) is a novel multi-function proinflammatory factor (1). Our previous studies and studies from several other groups revealed that IL-18 was involved in and aggravated the progress of many primary and secondary kidney diseases (2-7). IL-18 binding protein (IL-18BP) is a circulating protein with high affinity for IL-18 and can neutralize its bioactivity, known as IL-18 natural antagonist (8, 9). Our previous studies showed that plasma level of IL-18 was increased significantly in patients with lupus nephritis (LN) while not increased in primary nephrotic syndrome (PNS) with normal renal function (3, 10). The plasma level of IL-18BP was also reported elevation in many human diseases, including acute and chronic renal failure (11). But the secretion levels of IL-18BP in lupus nephritis (LN) and PNS are still unclear. We inferred that if the levels of IL-18 and IL-18BP elevate parallel in a disease, circulating IL-18 may not exert its biological activity in that disease, basing on not increasing free IL-18 concentration in circulation. Up-to-date, the balance between IL-18 and IL-18BP in patients with LN and PNS is still unknown. In the present study, plasma levels as well as mRNA expression in peripheral blood mononuclear cells (PBMCs) of IL-18 and IL-18BP were measured in 20 patients with PNS, 16 patients with LN and 11 healthy volunteers. The balance status of IL-18/IL-18BP was then evaluated. The aim of the present study is to investigate whether IL-18BP can be act as a possible therapeutic approach against the two kidney diseases.

Materials and Methods

Patients and controls

Twenty patients with PNS (13 males and 7 females), 16 patients with LN (1 male and 15 females) and 11 healthy volunteers (6 males and 5 females) were enrolled in this study. The criterion of PNS diagnosis includes urinary protein excretion quantity more than 3.5 g/d, level of plasma albumin less than 30 g/L, and accompanying with or without severe edema and/or hypercholesterolemia. Renal biopsy was performed to further confirm the diagnosis and excluded secondary nephrotic syndrome. The renal function of all PNS patients recruited in this study is normal. The diagnosis of systemic lupus erythematosus was confirmed according to the criterion of American Rheumatism Association, and all the patients were confirmed the present of active renal lesion by urinary examination and renal biopsy. Patients of PNS and LN had never received any immunorepressive agents and dialysis before being recruited in the study.

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Table 1. Polymerase chain reaction (PCR) primer sequences, amplification condition and product size

Primer		Sequence	Condition	Size
IL-18	F	5′ GCTTGAATCTAAATTATCAGTC 3′	94°C 1 min, 55°C 1 min, 72°C 1min, 32 cycles	342 bp
	R	5′ GAAGATTCAAATTGCATCTTAT 3′		
IL-18BPa	F	5′ TGGGAGATGTAGCCGACCTT 3′	94°C 45 s, 57 °C 45 s, 72°C 1 min, 32 cycles	466 bp
	R	5′ GCCCCGACCCTCTATTTCA 3′		
GAPDH	F	5′ TGGTATCGTGGAAGGACTCATGAC 3′	94°C 1 min, 55°C 1 min, 72°C 1 min, 28 cycles	189 bp
	R	5′ ATGCCAGTGAGCTTCCCGTTCAGC 3′		

Semi-quantitative RT-PCR

Heparin anticoagulant blood (8 ml) was extracted from each subject. Plasma was isolated and stored at -70°C for latter enzyme-linked immunoadsordent assay (ELISA) experiment. PBMCs were isolated by density gradient centrifugation from the residual-precipitated blood cells. Total RNA was isolated from PBMCs using TRIzol reagent (Introvigen) according to the manufacturer's protocol. cDNA was synthesized by extension of Oligo (dT) 12-18 primers using Superscript™ Amplification System (Introvigen) according to the protocol of manufacturer. The data of primers sequence, amplification conditions and length of each product were collected in Table 1. PCR amplified system included 2 μl 10× PCR buffer, 1.2 μl 25 mmol/L $MgCl_2$, 0.4 μl 10 mmol/L dNTP mix, 0.4 μl 10 mmol/L sense and anti-sense primers respectively, Taq DNA polymerase 2 U, cDNA 1 μg and H_2O to total 20 μl. PCR was performed in a thermal cycler (Eppendorf, New York, USA). Each target gene product was mixed with equal volume of control housekeeper gene product and then performed electrophoresis. A gel imaging system (UVP, Cambridge, UK) was used to evaluate semi-quantitatively the integral optical density (IOD) of each amplified band. Expression quantity of each target gene was represented as ratio of IOD of the target gene divided by IOD of a housekeeper gene in each sample.

Enzyme linked immunosorbent assay

Plasma levels of IL-18 were determined by a commercial human IL-18 ELISA kit (Huamei, Beijing, China) according to the protocol of manufacturer. The detection limit is 12.5 pg/ml. The kit for IL-18BPa quantity was coated by the authors using specific monoclonal antibody. Briefly, 100 μl of monoclonal IL-18BPa antibody (R&D System, MN, USA) solution (2.0 μg/ml in PBS, pH 7.2) was added into each well in a 96-well microplate, and the microplate was incubated at room temperature overnight, then washed with washing solution 5 times. After that, 300 μl of 1% fetal bovine serum in PBS was added and the plate was incubated for another 1 h in room temperature. The microplate was washed again for 5 times. The secondary antibody used in this ELISA was biotinylate goat antibody (0.2 μg/ml, R&D system) and the substrate was Streptavidin-HRP (LIVZON Pharmaceutical Group Inc., Zhuhai, China). Plasma was 1:10 diluted before assay. The ELISA procedure was described elsewhere. OD was read at a micro-plate reader (Bio-Rad, CA, USA) at 450 nm at last.

Statistical analysis

Statistical analysis was performed using SPSS 12.0 version software. The data were expressed as the mean ± SD. Statistical significance was assessed by one way ANOVA and followed by Bonferroni or Tambane's post Hos multiple comparisons.

Results

Protein secretion levels

Plasma level of IL-18 was increased significantly in LN group ($p < 0.05$), while not increased significantly in PNS group when compared with that in normal group. Plasma level of IL-18BP was increased significantly in LN group ($p < 0.05$) as well as in PNS group ($p < 0.01$) when compared with that in normal group. The ratio of IL-18/IL-18BP in LN group was increased significantly when compared with that of normal group ($p < 0.005$), but there was no significant difference between PNS group and normal group (Table 2).

mRNA expression

The level of IL-18 mRNA expression in PBMCs in LN group was significantly higher than that in normal group ($p < 0.001$), while there was no statistical difference between PNS group and control group. The level of IL-18BP mRNA expression in PBMCs in LN group as well as in PNS group was higher than that in control group ($p < 0.05$). The ratio of IL-18 mRNA/IL-18BP mRNA in LN group has a trend of elevation, but not reaching statistic significance when compared with that of control group. The ratio of IL-18 mRNA/IL-18BP mRNA was not significantly different between PNS group and control group (Table 3 and Figure 1).

Discussion

It is well known that LN is an organ non-specific autoimmune disease and the characteristic of this disease is the production of numerous antibodies and immune complex. Immune disorder, particular abnormal secretion of numerous of cytokines is the central mechanism of LN. PNS is a

Table 2. Plasma level of IL-18 and IL-18BP, and the ratio of IL-18/IL-18BP in normal control, patients with PNS and patients with LN

Groups	n	IL-18 (pg/ml)	IL-18BP (ng/ml)	IL-18/IL-18BP
Control	11	238.9 ± 64.4	5.6 ± 2.1	0.052 ± 0.033
PNS	20	289.0 ± 70.5	8.5 ± 3.4*	0.048 ± 0.051
LN	16	767.0 ± 133.5*	8.0 ± 3.0**	0.113 ± 0.060#

*$p < 0.01$, **$p < 0.05$, #$p < 0.005$, compared with control.

Table 3. mRNA expression of IL-18 and IL-18BP, and the ratio of IL-18 mRNA/IL-18BPa mRNA in PBMCs in normal control, patients with PNS and patients with LN

Groups	n	IL-18 mRNA	IL-18BP mRNA	IL-18 mRNA/IL-18BP mRNA
Control	11	0.70 ± 0.34	0.69 ± 0.22	1.38 ± 1.45
PNS	20	0.90 ± 0.38	0.87 ± 0.35	1.23 ± 0.72
LN	16	1.57 ± 0.34*	0.97 ± 0.30**	2.10 ± 2.09

*$p < 0.001$, **$p < 0.05$, compared with control.

primary glomerulonephropathy of various pathological manifestations. PNS is also an immune inflammatory disease and numerous cytokines abnormal secretion during the initiation and progress of this disease. In a word, cytokine network imbalance plays a crucial role in the initiation and progress of LN and PNS. IL-18 is a novel multi-function proinflammatory cytokine. It is the strong inducer of IFN-γ and also can promote production of many other inflammatory mediators, such as tumor necrosis factor-α, granulocyte-marcrophage colony stimulating factor (GM-CSF) and Fas ligand (1). Large number of studies showed that IL-18 plays an important role in many immune inflammatory diseases, including in primary and secondary glomerulonephropathies (2-7).

Our previous study demonstrated that levels of IL-18 in patients with LN were several-fold higher than those of normal control, and renal gene and protein expression of IL-18 in these patients were also increased significantly, which was also well correlated with LN renal histological activity index (AI) (3). We have also reported that plasma level of IL-18 was normal, while the expression of IL-18 in renal tissue and the excretion of IL-18 in urine were increased significantly (10, 12). From these results, we inferred that IL-18 was involved in the progress of LN *via* systemic and local approach, while IL-18 only *via* local approach in the progress of PNS.

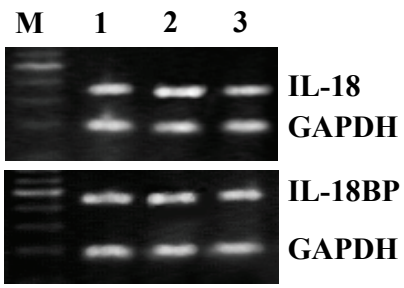

Figure 1. Expression of IL-18 and IL-18BP mRNA in patients with PNS, patients with LN and normal control. Lane M, 100 bp DNA ladder; Lane 1, PNS; Lane 2, LN; Lane 3, normal control.

The patients have not received immunosuppressors before recruited into this study. This guarantees the immune disorder has not been disturbed by outer factors. The present results approved our previous studies that plasma level of IL-18 and the mRNA expression of IL-18 of PBMCs were increased significantly in patients with LN, while the plasma level of IL-18 in PNS group was not increased significantly. These results were not the aim of the present study. The real aim of the present study is to investigate the balance of IL-18 and its natural antagonist, IL-18BP in these two kidney diseases.

IL-18BP was mainly produced by monocytes. There are 4 isoforms of IL-18BP in humans. IL-18BPb and IL-18BPd isoforms lack the ability to bind and naturalize IL-18. The affinity of IL-18BPc isoform is 10 fold less than IL-18BPa isoform. Taken together, IL-18BPa which was measured in this study is the main IL-18BP in humans (13). IL-18BPa has high affinity for IL-18 and the dissociate constant (Kd) is approximate 400 pM. IL-18BP exerts its function by binding and naturalizing the biological activity of IL-18 (9). Data showed that IL-18BP can inhibit the production of IFN-γ up to 90% induced by LPS in mice (8). On the other hand, IFN-γ is the most important inducer of IL-18BP (1). And as mentioned above, IL-18 is the strongest inducer of IFN-γ. Summarily, IL-18, IFN-γ and IL-18BP make up of a negative feedback network. Due to its special property, the role of IL-18BP has been investigated in many areas, but not yet in LN and PNS.

In the present study, plasma level and mRNA expression of IL-18BP in PBMCs were increased significantly in patients with PNS and LN. We inferred that IL-18BP was induced to increase directly by IL-18 or indirectly *via* IFN-γ and then to antagonist the action of IL-18. We found that the levels of IL-18 and IL-18BP in PNS were increased in parallel. In other words, level of free IL-18 was not increased in PNS. But in LN, the ratio of IL-18/IL-18BP was much higher than that in normal control. It means that both the total IL-18 increase and free IL-18 were increased in circulation in patients with LN. That is to say, the elevation of IL-18BP is not enough to counteract the elevation of IL-18 in LN. There were reports demonstrated that increasing the level of IL-18BP can alleviate the injury of liver and joints in patients with hepatitis C and animal model with collagen-induced arthritis (14, 15). We infer that regulating the balance of IL-18 and IL-18BP in LN may be also a therapeutic approach against LN thought further studies are needed.

Acknowledgements

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