

Effect of serum Th1/Th2 cytokine imbalance on the occurrence and development of various chronic glomerulonephritis.

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Abstract

Objective: To explore the changes of serum interferon- γ (IFN- γ) and interleukin-10 (IL-10) of various chronic glomerulonephritis (CGN) patients and further investigate the effect of serum cytokine Th1/Th2 on the occurrence and development of various CGN.

Methods: Venous blood of 9 patients with IgA mesangioproliferative glomerulonephritis (IgA nephropathy), 18 patients with non-IgA mesangioproliferative glomerulonephritis (non-IgA MsPGN), 7 patients with membranous nephropathy (MN), 6 patients with focal segmental sclerosing glomerulonephritis (FSGS), 14 patients with chronic renal failure, and 20 health people were collected for the study. Serum IFN- γ and IL-10 were assayed by double antibody sandwich ELISA, and there was a measurement of 24 h urinary protein excretion and serum creatinine which were statistically analysed.

Results: The IFN- γ of the patients with IgA nephropathy or non-IgA MsPGN saw a great growth, but that of the patients with MN badly reduced, and IFN- γ of the patients' non-IgA MsPGN was positively related to 24 h urinary protein excretion; IL-10 of the patients IgA nephropathy, non-IgA MsPGN, FSGC and CRF saw a significant decrease, and that of the patients with CRF was negatively associated with serum creatinine.

Conclusion: Serum IFN- γ plays a role in the improvement of immunologic injury of mesangioproliferative glomerulonephritis which is related to the formation of proteinuria; the occurrence and development of CGN is closely linked to the decrease of IL-10, so does the development of CRF, and the imbalance of serum TH1/TH2 cytokines has certain correlation with clinical indexes.

Keywords: Chronic glomerulonephritis, Th1/Th2 cytokine, Interferon- γ , Interleukin-10.

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Introduction

Chronic glomerulonephritis (CGN), chronic nephritis in short, is a common primary renal glomerular disease consisting of multi-factors and multi-pathological types [1]. The pathogenesis of CGN is kidney damage mainly caused by immune response rather than pathogen itself directly affecting the kidneys, whose clinical features are following: insidious onset, mild or severe symptoms, lingering and protracted illness (ranged from 1 year to decades), and most of them are slowly and progressively aggravated. Routine urine test usually shows proteinuria, haematuria, cylindruria, etc. What's more, the patients also have varying degrees of oedema, hypertension and renal damage. If the diagnosis and treatment are delayed, the severe one could develop into Chronic renal failure (CRF) because the health nephrons are ceaselessly and badly destroyed, and residue nephrons are gradually reduced [2,3]. According to the difference of pathological types, CGN can be divided into mesangioproliferative glomerulonephritis (including IgA mesangioproliferative glomerulonephritis and non-IgA mesangioproliferative glomerulonephritis), mesangial capillary glomerulonephritis (MCGN), membranous nephropathy (MN), and focal segmental sclerosing

glomerulonephritis (FSGS) [4]. CGN is an autoimmune systemic disease whose central part is immune-inflammatory reaction [5]. Researches have shown that there are Th1 and Th2 subsets of helper T cells in human body, and Th1 cytokines are mainly cellular immune response and secret interferon- γ (IFN- γ), while Th2 cytokines are generally humoral immune reaction and secret interleukin-10 (IL-10) [6]. The relationship of Th1 and Th2 are mutual regulation, namely the balance of Th1/Th2 function [7]. The imbalance of Th1/Th2 cytokines interfere the occurrence and development of various immune response diseases [8]. The major pathogenesis of CGN is renal-inflammatory damage due to disorder of immune system, but it is also related to the imbalance of Th1/Th2 cytokines [9,10]. And there is a big difference in the prognosis of CGN which may be caused by its various clinical manifestations and organizational changes [11]. However, there are few studies on the imbalance of serum Th1/Th2 cytokines and its significance in China. In the study, we systematically detect the expression of IFN- γ and IL-10 in serum of the patients with various CGN and synthetically analyse their clinical indexes to have an idea of the features of cytokine balance changes and immune state of the patients,

which provides a scientific basis for the study on its pathogenesis, and diagnosis, treatment, and prognosis in clinic.

Materials and Methods

General materials

CGN group: there were 40 patients, who were in accordance with the clinical differentiation standards of primary renal glomerular disease drafted by the editorial board of Chinese Journal of Internal Medicine in 1992 [12], 19 males, 21 females, with a mean age of (39.04 ± 12.35) years. If patients had secondary renal disorders such as renal damage due to multiple myeloma, diabetic nephropathy, purpura nephritis, and hypertensive nephropathy, and if patients had liver and kidney dysfunction or other immune diseases, they would be ineligible for the study. All patients in the study hadn't been treated by such drugs like immunosuppressant and glucocorticoid for six months at least. CRF group: there were 14 patients with CRF, 6 males, 8 females, with an average age of (42.16 ± 19.74) years, whose primary diseases were chronic glomerulonephritis and serum creatinine over $144 \mu\text{mol/L}$, and they all hadn't been treated by peritoneal dialysis or haemodialysis. Control group: there were 20 health people, 9 males, 11 females, with a mean age of (35.47 ± 3.85) , whose blood fat, liver function, and kidney function were normal. All participants voluntarily joined in the study and carefully read and signed the informed consent form.

Renal biopsy

In the CGN group, the patients need have coagulation tests before renal biopsy to ensure their clotting function within the normal range. The renal biopsy must be conducted using vacuum aspiration method performed by two individuals with the guidance of ultrasound. About 4 mm of the cortical end of the renal tissues obtained was placed in gauze infiltrated with normal saline, and the remaining portion was fixed with 10% formaldehyde solution, stored in an ice box at about 4 for light microscopic examination and immunofluorescence in 24 h, so as to determine the pathological type of CGN (the pathological types conformed to the pathological typing scheme published by the WHO in 1995) [13].

Collection and storage of samples

All patients and the health people were collected 5 ml venous blood with anticoagulation which were centrifuged at 3000 RPM for 10 min and the supernatant discarded, stored at -80°C . All samples were exposed to a freeze/thaw only once before assaying IFN- γ and IL-10.

In addition, the patients with CGN or CRF were collected 2 ml venous blood without anticoagulation at the same time for testing serum creatinine timely, and their 24 h urine samples were quantified for proteinuria.

Detection methods

Detection of the expressions of interferon- γ and interleukin-10 in serum: The expressions of IFN- γ and IL-10 in serum of the patients in two groups were detected by double antibody sandwich ELISA, and the detection wavelength of microplate reader was 450 nm.

Measurement of 24 h urinary protein excretion: The measurement of 24 h urinary protein excretion of the patients was detected by turbidimetry.

Detection of serum creatinine: Picric acid colorimetry was used for the detection of serum creatinine.

Statistical analysis

All data were analysed by software SPSS18.9, and the results of detection were expressed by mean \pm SD. Homogeneity test for variance was conducted before comparison. Student-Newman-Keuis was used for the comparison on means of each CGN group with various pathological types and the control group, while t-test was used for the comparison on means of the CRF group and the control group. The heterogeneity of variance was analysed with the method of t test, while the correlation was analysed by linear correlation regression. $P < 0.05$ was defined as significant difference.

Results

Pathological typing of chronic glomerulonephritis

40 CRF patients were divided into four pathological types through immunofluorescence and optical microscopy (Table 1).

Table 1. Pathological typing of CGN.

Pathological typing amount	(Case)
IgA mesangioproliferative glomerulonephritis (IgA nephropathy)	9
Non-IgA mesangioproliferative glomerulonephritis (non-IgA MsPGN)	18
Membranous nephropathy (MN)	7
Focal segmental sclerosing glomerulonephritis (FSGS)	6

Comparison on the level of IFN- γ in serum of each pathological type and the control group

The levels of IL-10 in serum of the IgA nephropathy group and the non-IgA MsPGN group were increased much more than that of the control group ($P < 0.01$), while compared the IgA nephropathy group and the non-IgA MsPGN group, there was no difference in serum IFN- γ level ($P > 0.05$); the serum IFN- γ level of the MN group was much less than that of the control group ($P < 0.01$); when compared the FSGS group and the

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control group, there was also no statistical difference ($P>0.05$) (Table 2).

Table 2. Comparison on the level of IFN- γ in serum of each pathological type and the control group ($\pm s$, pg/ml).

Group	N	IFN- γ
IgA nephropathy group	9	27.57 \pm 1.83**
Non-IgA MsPGN group	18	23.43 \pm 4.59**
MN group	7	7.52 \pm 2.36**
FSGS group	6	12.73 \pm 3.02
Control group	20	13.75 \pm 1.26

Note: compared with the control group, ** $P<0.01$

Comparison on the level of IL-10 in serum of each pathological type and the control group

The levels of serum IL-10 of the IgA nephropathy group, non-IgA MsPGN group, and FSGS group were much less than that of the control group ($P<0.01$), while compared these three nephropathy groups, there was no difference in the level of serum IL-10 ($P>0.05$); what's more, compared the serum IL-10 levels of the MN group and the control group, there was no significant difference ($P>0.05$) (Table 3).

Table 3. Comparison on the level of IL-10 in serum of each pathological type and the control group ($\pm s$, pg/ml).

Group	N	IL-10
IgA nephropathy group	9	10.47 \pm 1.63**
Non-IgA MsPGN group	18	10.33 \pm 2.72**
MN group	7	16.52 \pm 2.77
FSGS group	6	7.79 \pm 2.67**
Control group	20	16.29 \pm 3.29

Note: compared with the control group, ** $P<0.01$

Comparison on the levels of IFN- γ and IL-10 in serum of the chronic renal failure group and the control group

The level of IL-10 in serum of the CRF group was much lower than that of the control group ($P<0.01$), while compared two group, there was no statistical difference in the level of IFN- γ ($P>0.05$) (Table 4).

Table 4. Comparison on the levels of IFN- γ and IL-10 in serum of the chronic renal failure group and the control group ($\pm s$, pg/ml).

Group	N	IFN- γ	IL-10
CRF group	14	12.17 \pm 1.52	8.31 \pm 0.42**
Control group	20	11.55 \pm 2.79	18.29 \pm 1.29

Note: compared with the control group, ** $P<0.01$

The correlation between the levels of IFN- γ and IL-10 of each pathological type and the measurement of 24 h urinary protein excretion

The level of serum IFN- γ in the non-IgA MsPGN group was positively correlated with 24 h urinary protein excretion ($r=0.76$, $P<0.01$), while that of the other three groups had no correlation with it ($P>0.05$). However, there was no correlation between level serum IL-10 and serum protein level in the four pathological types ($P>0.05$).

The correlation between the levels of IFN- γ and IL-10 in serum of the patients with CRF and the concentration of serum creatinine

In the CRF group, the level of IL-10 in serum was negatively associated with the concentration of serum creatinine ($r=-0.82$, $P<0.01$); while that of the IFN- γ in serum had no correlation with serum creatinine ($P>0.05$).

Discussion

Abnormal immune responses such as cellular immunity and humoral immunity are the major pathogenesis of CGN. IFN- γ plays a key role in cellular immunity which can give rise to renal damage. While serum Th1 cytokine mainly secretes IFN- γ of which growth can make it dominate the condition of inflammation [14]. IFN- γ intervenes MHC antigen and has its expression increased, and also directly leads to the immune damage of kidney tubules and glomerulus [15]. The level of IFN- γ in the supernatant of peripheral mononuclear cells of the patients with mesangial proliferative glomerulonephritis is much higher than that of the health people, which is consistent with the content of renal tissue, and the growth of IFN- γ of peripheral mononuclear cells is also accordance with the degree of renal damage [16]. In the study, the levels of serum IFN- γ of the IgA group and non-IgA MsPGN see a significant upward trend, and that of the non-IgA MsPGN is positively correlated with urinary protein, which may be related to the increase of urinary protein filtration. Serum Th2 mainly secretes IL-10 involved in humoral immunity which is a vital immune regulatory factor that can control the function of Th1 cytokine. IL-10 may be another major anti-inflammatory drug apart from glucocorticoid [17]. An animal experiment conducted by Kitching AR testifies that the treatment for IL-10 can greatly reduce the infiltration of macrophage in the renal tissue of the MsPGN experimental model mice, but also inhibit the proliferation of mesangial cells [18]. The findings of this study reveal that the levels of serum IL-10 of the IgA group, non-IgA MsPGN group, and FSGS group are much lower than that of the health persons, and that of the CRF group is negatively correlated with the concentration of creatinine. Serum Th1 and Th2 cytokines are mutually regulated in immune responses, and the massive production of Th1 cytokine and its release can result in the inhibition of Th2 cytokine immune responses, and vice versa. Normally, serum

Th1 and Th2 cytokines are in equilibrium in the body. If one of them has immune shifting, it will trigger imbalance of Th1/Th2, which gives rise to many diseases, therefore, it is necessary to keep the relative equilibrium of serum Th1/Th2 cytokines in the body.

In a conclusion, the changes of TH1/Th2 cytokine balance of the chronic glomerulonephritis patients with various pathological types are different, and serum IFN- γ as well as IL-10 have definite correlation with clinical indexes like proteinuria and kidney function, so they can be regarded as the serum immunological indexes used for assessing renal damage in future.

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