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Original Research Article

Expressions of toll-like receptors 2 and 4, and relative cellular factors in HIV patients with tuberculosis infection

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Abstract

Purpose: *To investigate the expressions of toll-like receptor 2 (TLR2), toll-like receptor 4 (TLR4), tumor necrosis factor alpha (TNF-α), IFN-γ (IFN- gamma), interleukin 2 (IL-2), interleukin 6 (IL-6) and interleukin 10 (IL-10) in human immunodeficiency virus (HIV) patients with tuberculosis (TB) infection.*
Methods: *Two groups of HIV patients (68 in each group) were used for this study. These were HIV with TB (HIV/TB) group and HIV without TB group. A third group (68 healthy people) served as control. Quantitative polymerase chain reaction (qPCR) was adopted to measure TLR-2 and TLR-4 expressions in peripheral blood mononuclear cells (PBMC), while the serum levels of TNF-α, IFN-γ, IL-2, IL-6 and IL-10 were determined by ELISA.*
Results: *The ∆Ct values of TLR-2 and TLR-4 in HIV/TB and HIV groups were significantly lower than those in the control group ($p < 0.05$). Compared to control group, the serum levels of TNF-α, IL-6 and IL-10 significantly increased, while IFN-γ and IL-2 in HIV/TB and HIV groups significantly decreased ($p < 0.05$). However, IFN-γ and IL-2 decreased significantly in HIV/TB group ($p < 0.05$). Expression of TLR2 correlated positively with serum levels of TNF-α, IL-6 and IL-10, but negatively with IFN-γ and IL-2 ($p < 0.05$).*
Conclusion: *TLR2 signal pathway plays a role in HIV patients with TB infection by promoting the expressions of TNF-α, IL-6 and IL-10, while inhibiting IFN-γ and IL-2 cellular factors, and thus may provide a new pathway for the treatment of patients with HIV/TB.*

Keywords: *HIV, Tuberculosis, Toll-like receptor, Cellular factors, Tumor necrosis factor, Interleukin*

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INTRODUCTION

In recent years, the incidence of HIV with TB infection has gradually increased with increasing cases of HIV infection. According to statistics, one-third of 34 million HIV infected patients world-wide have TB [1]. An investigation in South Africa showed that about half of 12,672 TB patients studied were infected with HIV [2]. HIV infection makes patients more susceptible to *Mycobacterium tuberculosis* (MTB) infection, and is the most important factor for causing TB. HIV infection can damage the human immune system, and cause potential TB to become active TB. HIV with TB infection is not superimposition of independent infectious processes and effects of two pathogens. The two are synergistic, thereby resulting in deterioration of the disease conditions to epidemic proportions.

Toll-like receptors (TLRs) are expressed mainly in immune cells, especially in mononuclear cells or macrophages, and on the surface of dendritic cells. They are a group of very important pattern recognition receptors of immunity, which can

recognize pathogen-associated molecular patterns (PAMPs) and activate *in vivo* inflammatory responses. They are the first line of defense in immune reactions [3]. In addition, TLRs can induce activation of T-lymphocytes, regulate and control adaptive immunity, and maintain balance of body immune system [4]. Studies have shown that TLR2 and TLR4 participate in the recognition of TB and associated metabolites, and promote inflammatory responses against them [5,6].

The present study was carried out to investigate the expressions of TLR2, TLR4, TNF-α, IFN-γ IL-2, IL-6) and IL-10) in HIV patients with TB infection

EXPERIMENTAL

General information on patients

Sixty-eight (68) HIV cases with TB infection (HIV/TB group) in our hospital who were given treatment from January, 2014 to December, 2016, were recruited in this study. They consisted of 49 males and 19 females, with ages ranging from 19 to 63 years (mean age = 37.82 ± 8.43 years). The control group was made up of 68 healthy individuals (50 males and 18 females) selected after medical examination. Their ages ranged from 20 to 65 years (mean age = 37.46 ± 8.71 years). Diagnostic standards of HIV conformed with the *AIDS Diagnostic and Treatment Guide, 2011 Edition* [7]. Diagnosis of TB was in line with the diagnostic standards of *Diagnostic Criteria for Pulmonary Tuberculosis (WS288-2008)* by National Health and Family Planning Commission of the People's Republic of China [8]. The inclusion criteria in the control group were: absence organ diseases of heart, brain, lung, autoimmune system and connective tissue. In addition, the selected patients were those who did not receive immunosuppressant drugs or anti-TB treatment within the previous year, and those who were free from tumor, severe infection, and other immunodeficiency diseases. The Medical Ethics Committee of The Chest Hospital of Linyi, Shandong Province, China approved this study (no. LY2014005), and which followed the Helsinki Declaration [9]. All subjects agreed to participate in this study and signed informed consent. Comparison of baseline information between three groups did not reveal any significant differences ($p > 0.05$).

Sample collection

Peripheral blood (10 mL) was collected under sterile conditions and divided into two, 5-mL portions. To one portion was added 3.8 % sodium citrate, and PBMCs was separated by Ficoll lymphocyte separation medium (America Sigma company). Total RNA in PBMCs was extracted with Trizol reagent (America Invitrogen company), and cDNA was synthesized by reverse transcription (America Promega Company). The cDNA was stored at – 20 ^{o}C. The second 5 mL portion of blood was centrifuged at 3000 r/min for 10 min to obtain serum which was stored in a fridge at – 20 ^{o}C prior to analysis.

Biochemical assays

Polymerase chain reaction (qPCR) was used to measure the expressions of TLR2 and TLR4 mRNA. Primer design was synthesized by Shanghai Biotechnology. The internal control was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers used are shown on Table 1. The 20 μL reaction mixture included 10 μL of 2 × PCR buffer solution, 0.5 μL of upstream and downstream primers, 2 μL of cDNA and 7 μL of sterilized distilled water. Reaction conditions: pre-degeneration was at 94 ^{o}C for 30 s; PCR reaction was at 94 ^{o}C for 10 s, 60 ^{o}C for 20 s, 40 circles. Curve analysis of solution was at 70~90 ^{o}C /s and reading value was 1s. Gene amplification was quantified by using $2^{-\Delta\Delta ct}$ method. The level of TNF-α, IFN-γ, IL-2, IL-6 and IL-10 in serum were determined by ELISA kits (America R&D Company).

Statistical analysis

SPSS 21.0 software was used to analyze all data, and are presented as mean ± standard deviation (SD).

Table 1: Premier sequence and amplification segments of TLR2, TLR4 and GAPDH

Primer	Sequence	(bp)
TLR2-F	5'-CCAAGAGGAAGCCCAAGAAAG-3'	154
TLR2-R	5'-AAGTCCCGCTTGTGGAGACAC-3'	
TLR4-F	5'-TTGAGCAGGTCTAGGGTGATTGAAC-3'	143
TLR4-R	5'-ATGCGGACACACACACTTTCAAAT-3'	
GAPDH-F	5-GCACCGTCAAGGCTGAGAAC-3'	138
GAPDH-R	5'-TGGTGAAGACGCCAGTGGA-3'	

Comparison between the three groups were done by ANOVA. Comparisons between two groups were analyzed by t-test. Enumeration data were analyzed and compared using χ^2 test. Correlation analysis between double-variables was done by Pearson correlation analysis. $P < 0.05$ was considered statistically significant difference.

RESULTS

Compared with HIV/TB group, the expressions of TLR-2 and TLR-4 mRNA△C values in the control group and HIV group were significantly higher ($p < 0.05$). In addition, TLR-4 mRNA△C value in the HIV/TB group was significantly lower than that in HIV group ($p < 0.05$). The levels of TLR-4 mRNA△Ct between the HIV/TB group and HIV group showed no significantly differences ($p > 0.05$, Table 2).

When compared with the control group, the serum levels of TNF-α, IL-6 and IL-10 in HIV/TB and HIV groups were significantly increased while the levels of IFN-γ and IL-2 decreased significantly ($p < 0.05$). In addition, compared with HIV group, the serum levels of TNF-α, IL-6 and IL-10 in HIV/TB group were significantly increased but IFN-γ and IL-2 were significantly decreased ($p < 0.05$, Table 3).

Correlation analysis (Table 4) showed that TLR2 expression was positively in correlation with the levels of TNF-α, IL-6 and IL-10, and negatively correlated with the levels of IFN-γ and IL-2 ($p < 0.05$). However, the TLR4 expression was no obvious correlation with the serum levels of TNF-α, lFN-γ, IL-2, IL-6 and IL-10 ($p > 0.05$).

DISCUSSION

$CD4^+$T cells decrease constantly in HIV patients, resulting in steady decline in immunity, which leads to infections, activation of potential MTB *in vivo* and new TB cases. MTB infection promotes HIV viral multiplication thereby aggravating the immunodeficiency disease [10]. Thus HIV and MTB work synergistically to exacerbate the degree of infection and death rate in patients who have both conditions [11,12]. In recent years, studies on HIV with TB infection have increased, and some progress has been made in understanding the pathogenesis of this combined infection. However, the immune system of HIV patients with TB infection is rather complex. At present, there are differences in the understanding of the pathogenesis of HIV complicated with TB infection [13,14].

Table 2: Expressions of TLR2 and TLR4 mRNA in the three groups (mean ± SD, △Ct values)

Group	No. of cases	TLR2 mRNA	TLR4 mRNA
Control group	68	2.87±0.80	4.20±0.82
HIV group	68	2.08±0.61[a]	3.47±0.78[a]
HIV/TB group	68	1.69±0.57[ab]	3.31±0.72 [a]
F-value		55.15	25.52
P-value		0.00	0.00

[a] *Compared with the control group (p < 0.05);* [b] *compared with the HIV group (p < 0.05)*

Table 3: Serum levels of TNF-α, IFN-γ, IL-2、IL-6 and IL-10 in the three groups (pg/mL, mean ± SD)

Group	Cases	TNF-α	IFN-γ	IL-2	IL-6	IL-10
Control	68	22.54±6.02	38.42±7.15	26.84±7.36	77.62±13.68	0.64±0.17
HIV	68	41.83±9.64[a]	27.60±6.31[a]	19.28±5.52[a]	172.38±36.84[a]	2.57±0.67[a]
HIV/TB	68	53.13±10.97[ab]	22.78±6.30[ab]	14.28±5.37[ab]	283.82±51.43[ab]	4.83±0.70[ab]
F-value		195.62	100.19	71.88	518.65	927.06
P-value		0.00	0.00	0.00	0.00	0.00

[a] *Compared with the control group (p < 0.05);* [b] *compared with the HIV group (p < 0.05)*

Table 4: Correlations between TLR2, TLR4 expressions and the level of TNF-α, IL-6 and IL-10 in serum

Item	TLR2 mRNA		TLR4 mRNA	
	R value	*P value*	*R value*	*P value*
TNF-α	0.537	0.000	0.203	0.182
IFN-γ	-0.426	0.001	-0.035	0.705
IL-2	-0.525	0.000	-0.162	0.191
IL-6	0.648	0.000	0.078	0.484
IL-10	0.403	0.001	0.154	0.273

As pattern-recognition receptors (PRRs) of natal immunity, TLRs can recognize PAMPs of pathogenic microorganisms, activate congenital immune response reaction in innate immune response, and also trigger release of immune factors [15,16]. The results of this study show that the expressions of mRNAs of TLR2 and TLR4 in HIV patients with TB, and HIV patients without TB, are higher than the corresponding expressions in healthy people. This shows that HIV infection up-regulates TLR2 and TLR4 expressions. This finding is in agreement with previous reports [17,18]. The TLR-2 mRNA level of HIV patients with TB infection was higher when compared with its value in the HIV group, but there were no differences in TLR4 mRNA between the two groups. The HIV virus multiplies rapidly and destroys $CD4T^+$ cells constantly, thereby weakening the body immunity and accelerating the spread of TB infection. The expressions of TLR2 and TLR4 have direct relationship with HIV viral multiplication. TLR2 and TLR4 expressions are up-regulated after HIV infection. At the same time, high expression of TLR2 can promote HIV viral multiplication, but TLR4 expression has no such effect [19]. Therefore, TLR4 does not have any effects on HIV patients with TB, but TLR2 may play a key role in these patients.

Balance between Th1 and Th2 cells is important for regulation of the immune system. Their cellular factors are TNF-α, IFN-γ, IL-2, IL-6 and IL-10. Th1 cells induce cellular response reaction and inflammatory reaction, but Th2 cell promote immunity of body fluids and resist parasitic infections; these two types of cells maintain balance in the immune system [20]. HIV infection affects the levels of Th1 and Th2. When the condition of the HIV infected person worsens, it causes imbalance in the secretion of Th1 and Th2 factors [21,22]. The results of this study show that the serum levels of TNF-α, IFN-γ, IL-2, IL-6 and IL-10 in HIV patients with TB were different from the corresponding levels in HIV and the healthy controls. This shows that the secretion of TNF-α, IL-6 and IL-10 are closely in correlation with HIV and TB infection. The analysis showed that in HIV patients with MTB, TNF-α, IL-6 and IL-10d were increased, while the production of IFN-γ and IL-2 was hindered. Thus the body immune function is inhibited, which increase susceptibility to MTB.

TLRs cause complex changes which culminate in secretion of cellular factors. It is still unclear how TLR2 influences HIV and MTB infection. Mehto *et al* [23] reported that MTB and HIV-1 promote apoptosis in macrophages through the TLR2 signal pathway. In addition, Qin *et al* [24] found that the inhibitory immune reaction of TLR2 is damaged by inducing over-expression of regulatory T-cell (Treg), which causes viral infection. In this study, the serum levels of NF-α, IFN-γ, IL-2, IL-6 and IL-10 were positively in correlation with the expression of TLR2, but not with the expression TLR4. This shows that TLR2 works in a synergistic manner with cellular factors, and that the early immune responses induced by HIV and MTB, depend solely on TLR2.

CONCLUSION

Overall, the findings of this study have demonstrated that TLR2 signal pathway has an important impact on HIV and TB infections by promoting the expressions of TNF-α, IL-6 and IL-10 and inhibiting those of IFN-γ and IL-2. The results also show that TLR2 is an effective inhibitor of HIV-1 multiplication in PBMCs. Thus, the findings may be useful in developing new treatment strategies for HIV and TB infections.

DECLARATIONS

Acknowledgement

None.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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