Changes of Lymphocyte Subsets in Pulmonary Tuberculosis

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Research

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Abstract

Background and Aims

The host immune system plays an important role in the pathogenesis and defense mechanism of Mycobacterium tuberculosis (M*tb*). This study aimed to explore the changes of immune system in Pulmonary Tuberculosis (PTB) patients.

Methods

A total of 85 active PTB patients and 50 healthy adults were enrolled, fifty patients with community-acquired pneumonia (CAP) were enrolled as the positive control. Chest CT and lymphocyte subgroup counts in peripheral blood were measured in all participants.

Results

In the PTB group, the level of CD4 + and B cells and the ratio of CD4+/CD8 + cells were significantly increased, the frequency of NK cells was significantly decreased at the same time.

Conclusions

The manifestation of immune system was changed in PTB patients, the inflammation in the infected lesion was limited which decreased innate immune activation, and the increased of T and B cells responses as the main pathogen elimination method.

Introduction

Pulmonary Tuberculosis (PTB) is caused by Mycobacterium tuberculosis (M*tb*). The main methods of bacteriological examination are sputum smear acid-fast staining test, M*tb* in sputum culture and Genexpert detection technology, which can help to confirm the diagnosis of PTB[1,2]. However, although researches on tuberculosis have been conducted for many years, the pathogenesis is still not fully understood. As host immune status is an important factor affecting the outcome after M*tb* infection, this study was designed to further explore the changes of immune responses in PTB patients.

Materials And Methods

Patients

Between August 2018 and August 2019, a total of 85 active PTB cases admitted to the Nanjing Jiangbei people's hospital were included in this study as the PTB group. The median age of the patients was 45.6 ± 18.8 years (range: 17–91 y), with 24 females (28.2%) and 61 males (71.8%), PTB patients were diagnosed by laboratory markers and computed tomography(CT)-acquiring images according to Diagnostic Criteria for Pulmonary Tuberculosis in China (WS 288–2017). Fifty healthy individuals were

enrolled as normal controls (Control group) and 50 patients with community-acquired pneumonia (CAP) were enrolled as the positive control (CAP group) (Table 1).

Table 1
Baseline characteristics of groups

	Control	CAP	PTB
Age (y)	39.9 ± 11.4	54.1 ± 20.6	46 ± 19
m/f	25/25	25/25	24/61
pulmonary cavity	N	N	32/85
multiple pulmonary cavities	N	N	10/85
CD4 + T cells (%)	39.6 ± 7.4	41.9 ± 10.3	44 ± 9.3*
CD8 + T cells (%)	27 ± 7.6	26 ± 10.5	23.6 ± 8.7
ratio of CD4+/ CD8 + T cells	1.6 ± 0.6	2 ± 1.2	2.2 ± 1.5*
B cells (%)	10 ± 2.5	13.7 ± 6.3**	13.7 ± 6.8**
NK cells (%)	18 ± 9.8	14.4.±10.1	13.4.±7.9*
CRP (mg/l)	2.8 ± 2.6	78.3 ± 83.1**	40.7 ± 28.5**
*P < 0.05;**P < 0.01			
PTB (Pulmonary Tuberculosis), CAP(community acquired pneumonia),NK (natural killer) cells, CRP(C-reactive protein)			

The exclusion criteria were as follows: (1) positive pregnancy test in females, (2) received immunomodulatory treatment within six months, (3) co-infected with human immunodeficiency virus, (4) accompanied by thyroid dysfunction, autoimmune diseases or psychiatric conditions.

Methods

Peripheral blood samples (5 ml) and sputum samples (3 ml) were taken from all the study participants. The numbers of lymphocyte subsets were detected by flow cytometry (Beckman). Sputum smear acid-fast staining test, Mtb in sputum culture and Genexpert test were conducted on all sputum specimens. C-reactive protein (CRP) was detected by ELISA.

Statistical analysis

Results were reported as means ± SD. Statistical comparisons were made between two groups using independent samples t-test. The one-way ANOVA method was used for multiple comparisons. Data analysis was performed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL). Confidence interval was 95%.

Results

Baseline characteristics of the groups

There was no significant difference in average age among the PTB, CAP and control groups. Results of chest CT showed that pulmonary cavities were found in 32 (37.6%) PTB cases. The cavity ratio and the proportion of multiple cavities were significantly higher in the PTB group (Table 1)..

The level of CD4 + cells and the ratio of CD4+/CD8 + cells were significantly increased in the PTB group

The level of CD4 + cells was significantly higher in the PTB group than in the control and CAP groups (44 ± 9.3 vs. 39.6 ± 7.4, $p < 0.05$)(Table 1, Fig. 1A), while the level of CD8 + cells showed no significant difference among the groups (Table 1, Fig. 1B). The ratio of CD4+/CD8 + cells was significantly higher in the PTB group than control (2.2 ± 1.5 vs. 1.6 ± 0.6, $p < 0.05$) (Table 1, Fig. 1C)..

The expression of CD3-CD19 + cells was significantly increased in the PTB and CAP groups

CD3-CD19 + cells were selected as B cells. The result showed that the level of B cells was significantly increased in the PTB and CAP groups, and there was no Significant difference between PTB (13.7 ± 6.8 vs. 10 ± 2.5, $p < 0.01$) and CAP groups(13.7 ± 6.3 vs. 10 ± 2.5 ,$p < 0.01$)(Table 1, Fig. 1D).

The frequencies of CD3-CD56 + cells were significantly decreased in the PTB group

CD3-CD56 + cells were selected as natural killer (NK) cells. The result showed that compared with control, the level of NK cells was significantly lower in the PTB group, and there was no significant change in CAP group(13.4.±7.9 vs. 18 ± 9.8,$p < 0.05$) (Table 1, Fig. 1E).

The level of CRP was significantly increased in the CAP and PTB groups

The level of CRP was determined as an infection indicator. The result showed that CRP was elevated in the PTB (40.7 ± 28.5 vs. 2.8 ± 2.6, $p < 0.05$) and CAP groups (78.3 ± 83.1 vs.2.8 ± 2.6, $p < 0.05$). Between PTB and CAP groups, CAP group has higher CRP expression (Table 1, Fig. 1F).

Discussion

Given the lack of specificity in clinical manifestations and chest CT changes in PTB, it is difficult to differentially diagnose PTB from lung cancer, lung tuberosity or other lung diseases.

M*tb* is a parasitic bacterium. The host immune system plays an important role in the pathogenesis and defense mechanism of M*tb*, and the main immune response is cell-mediated immunity (CMI). Research has shown that the M*tb* strains could stimulate CD4 + T cell proliferation[3]. After infection, M*tb* initially activates innate immunity, then natural receptors on the surface of macrophages recognize M*tb* and produce phagolysosomes to finally clear the bacteria. During this process, the level of CD4 + T cells increases in the peripheral blood of patients, which in turn increases the ratio of CD4+/CD8 + T cells, resulting in immune system disorders that contribute to disease progression[4–7]. The results of this study supported the aforementioned conclusions, which confirmed that the cellular immune function of tuberculosis patients was up-regulated.

The role of B cells in immunity against PTB remains controversial. Some scholars believed that most of the studies on PTB patients examined B cells isolated from peripheral blood and not inflamed M*tb*-affected tissues, which led to inconsistent conclusions on B cell levels in PTB[8]. There were experimental results to prove that inhibitory anti-PstS1 B cell responses arise during active tuberculosis[9]. The result of this study showed that the expression of B cells was higher in PTB. The possible reason is that after M*tb* infection, B cells proliferate and are accumulated at the site of pulmonary inflammation, where they secrete type I interferons to inhibit M*tb* proliferation and delay the migration of neutrophils to the lesion. In turn, the expression of B cells in peripheral blood is increased, which prompts the up-regulation of humoral immune function.

NK cells are the main effector cells of innate immunity, which possess potent cytolytic capacity without MHC-restriction. After attachment of M*tb* to the natural cytotoxicity receptor (NCR) NKp44 on NK cells, the cells can directly or indirectly control mycobacterial growth through cytotoxic mechanisms and macrophage activation[10]. Portevin D et al. found that NK cells were previously thought to be functionally impaired during TB[11]. This study proved that the expression of NK cells was significantly lower in PTB supported the conclusion and we speculate that due to the dysfunction of innate immunity, which led to decline in ability to inhibit the proliferation of M*tb* that resultes in colonization of tuberculosis bacteria in lungs, which in turn causes tuberculosis.

CRP is considered as an acute clinical indicator, and its level is often related to the severity of the disease. A previous study revealed a lower median CRP in PTB compared to bacterial pneumonia[12], and our study supported this conclusion. We found that the levels of CRP in PTB and CAP were much higher than the

control groupand CAP group has much higher level of CRP than PTB. Since CRP is a component of the innate immune response, it suggests that the innate immunity factors in PTB patients is not as strong as CAP.

Conclusion

PTB is easily confused with other pulmonary diseases. The number and virulence of bacteria, host immunity and other factors determine the outcome of the disease. This study demonstrated that the manifestation of immune system in PTB was different from healthy adults and CAP, the inflammation in the infected lesion was limited which decreased innate immune activation, and the increased of T and B cells response as the main pathogen elimination method.

The small sample population and short duration of follow-up were the limitations of this study. In this study, the immunomodulatory effects of M*tb* are preliminarily discussed, but the exact mechanism remains unknown. Hence, we will further investigate the pathways related to innate immune system and M*tb* infection, and delineate the phenotypic and functional characterization of immune cells.

Abbreviations

PTB Pulmonary Tuberculosis

CAP community-acquired pneumonia

NK cells natural killer cells

CRP C-reactive protein

Declarations

Ethical Approval: The study protocol was approved and monitored by the ethics committee of Nanjing Jiangbei Peoples' Hospital, and written informed consent was obtained from the patients

Consent for publication: All authors consent to publish

Availability of data and material: Our data in the manuscript available

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Authors' contributions: Hao Feng AB carried out the design of the study and performed the statistical analysis. Xue Yang carried out the immunoassays and drafted the manuscript.

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Figures

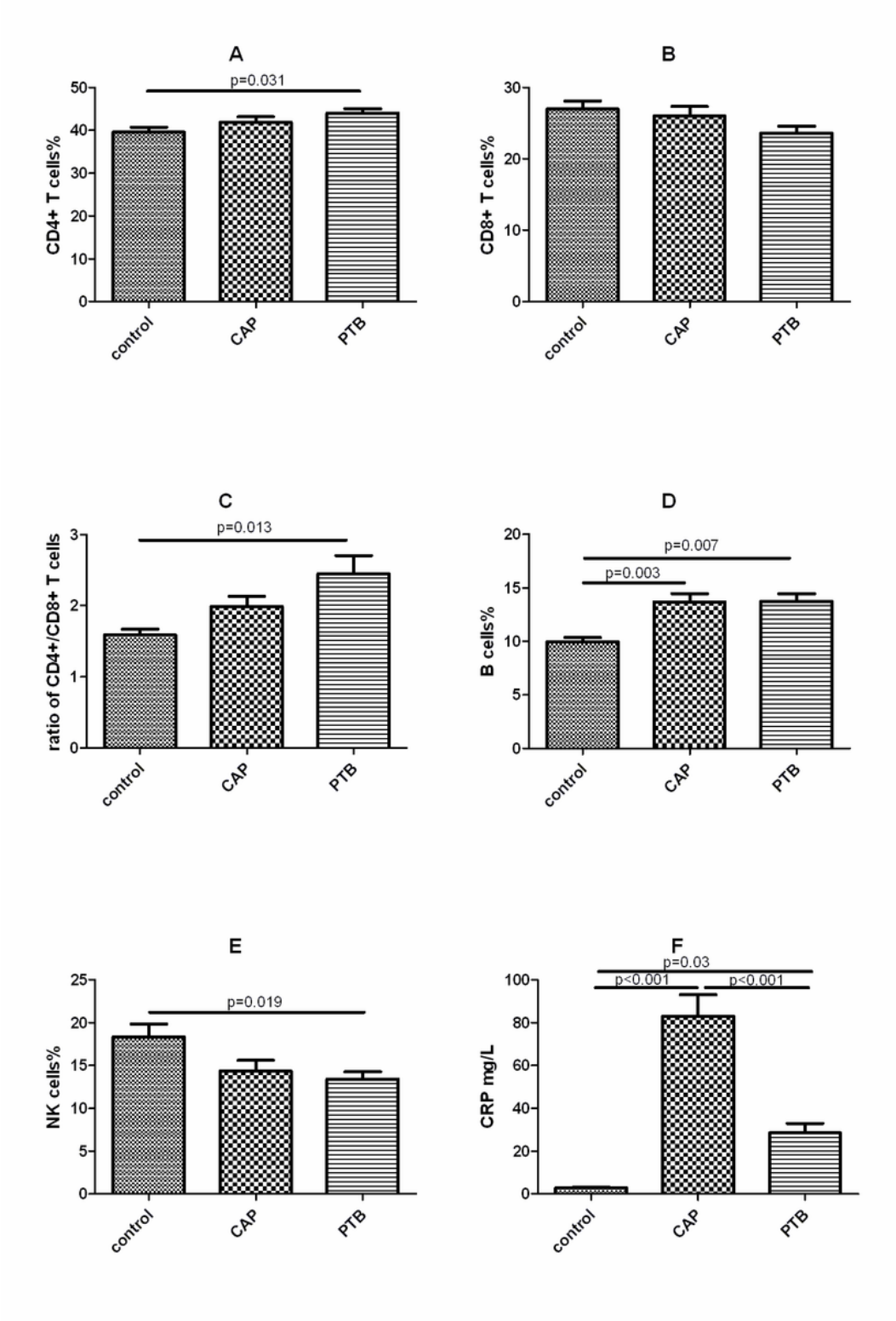

Figure 1

The number of lymphocytes in the various groups. The number of CD4+ T cells and the ratio of CD4+/CD8+ T cells were significantly higher in the PTB group than in the control and CAP groups (A, C). The number of CD8+ T cells showed no difference among the groups (B). B cells were significantly higher in the PTB and CAP groups (D). NK cells were decreased in the PTB group (E). CRP was increased in the

CAP and PTB groups compared to the control group (F). Abbreviations: PTB (Pulmonary Tuberculosis), CAP (community-acquired pneumonia), NK (natural killer) cells, CRP (C-reactive protein).