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Human Leukocyte Antigen-DR Expression in Peripheral Blood Mononuclear Cells in Patients with Tuberculosis

Chien-Ming Chu[1], Kuo-Chin Kao[2,3], Meng-Jer Hsieh[2,4], and Huang-Pin Wu[1,2]

[1]Division of Pulmonary, Critical Care and Sleep Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan;
[2]Chang Gung University College of Medicine, Taoyuan, Taiwan;
[3]Department of Thoracic Medicine, Chang Gung Memorial Hospital, Linkou, Taiwan;
[4]Department of Pulmonary and Critical Care Medicine, Chang Gung Memorial Hospital, Chiayi, Taiwan

Abstract

Immune response are necessary in the body's defense against tuberculosis. However, the activation of peripheral blood mononuclear cells (PBMCs) in patients with tuberculosis are still unclear. The aim of this study was to determine the pattern of activated monocytes and T lymphocytes in patients with tuberculosis. Twenty-seven healthy controls and 18 patients with newly diagnosed tuberculosis were enrolled. Human leukocyte antigen (HLA)-DR expression in monocytes, CD4 T lymphocytes, and CD8 T lymphocytes was measured using flow cytofluorimetry. CD8 T lymphocyte percentage in PBMCs and circulatory HLA-DR$^+$ CD8 T lymphocytes in patients with tuberculosis were lower than that in controls. HLA-DR expression in HLA-DR$^+$ monocytes and CD4 T lymphocytes in patients with tuberculosis was lower than that in controls. In conclusions, *Mycobacterium tuberculosis* infection might influence the activation of monocytes as well as CD4 and CD8 T lymphocytes. (J Intern Med Taiwan 2019; 30: 273-279)

Key Words: Monocyte, CD4 lymphocyte, CD8 lymphocyte, Human leukocyte antigen-DR, Tuberculosis

Introduction

Cellular immunity against pathogens is essential for humans. There is substantial evidence of the importance of CD4 T lymphocytes in controlling tuberculosis, but the role and activation of CD8 T lymphocytes in controlling this infection in humans remains unclear[1,2]. CD8 T lymphocytes are sometimes called cytotoxic T lymphocytes due to their ability to kill target cells. Cho et al. found that CD8 T lymphocytes derived from human blood can lyse *Mycobacterium tuberculosis* (*M. tuberculosis*)-infected macrophages and kill the intracellular bacilli[3]. CD8 T lymphocytes also play a critical role in controlling chronic viral infections; the role of these cells in containing chronic *M. tuberculo-*

Reprint requests and correspondence：Dr. Huang-Pin Wu
Address：Division of Pulmonary, Critical Care and Sleep Medicine, Chang Gung Memorial Hospital, Keelung. No.222, Maijin Rd., Anle District, Keelung, 204, Taiwan (R.O.C.)

Materials and Methods

Participants and definitions

Between August 2014 and July 2015, 18 patients with newly diagnosed pulmonary tuberculosis in a regional teaching and referral hospital were enrolled in this study. Patients with human immunodeficiency virus infection and those who was younger than 18 years were excluded. For comparison, 27 healthy controls were recruited from our Health Evaluation Center. All co-morbidities in subjects were recorded. Patients with tuberculosis had initial positive test results for acid-fast bacilli in sputum. Final results of sputum culture for *M. tuberculosis* after 6 weeks were positive in all 18 patients.

The Institutional Review Board of Chang Gung Memorial Hospital approved our study (100-4182B, 102-0128C). Healthy controls and patients with tuberculosis provided their written consent.

PBMCs preparation

Whole blood (20 mL) was collected at 08:30 AM in health controls; and at 08:30 AM in patients with tuberculosis within one week of starting anti-tuberculosis therapy. Samples were immediately heparinized. PBMCs were isolated via differential centrifugation over the Ficoll-Plaque (Amersham Biosciences, Uppsala, Sweden) within 2 hours of collection.

Immunophenotyping by flow cytometry

PBMCs at a density of 5 x 10^5 cells/tube were suspended in 50 µl of phosphate-buffered saline (PBS) and incubated in the dark for 15 min at room temperature with 10 µl of $CD4_{ECD}$, $HLA\text{-}DR_{FITC}$, $CD11b_{PC7}$, $CD8_{APC}$, $CD3_{Alexa\ Fluor\ 700}$, and $CD14_{APC\text{-}750}$ antibodies (Beckman Coulter, CA, USA). The cells were then resuspended in 500 µl of PBS. HLA-DR expression was detected using an eight-color flow cytofluorimeter (Beckman Coulter,

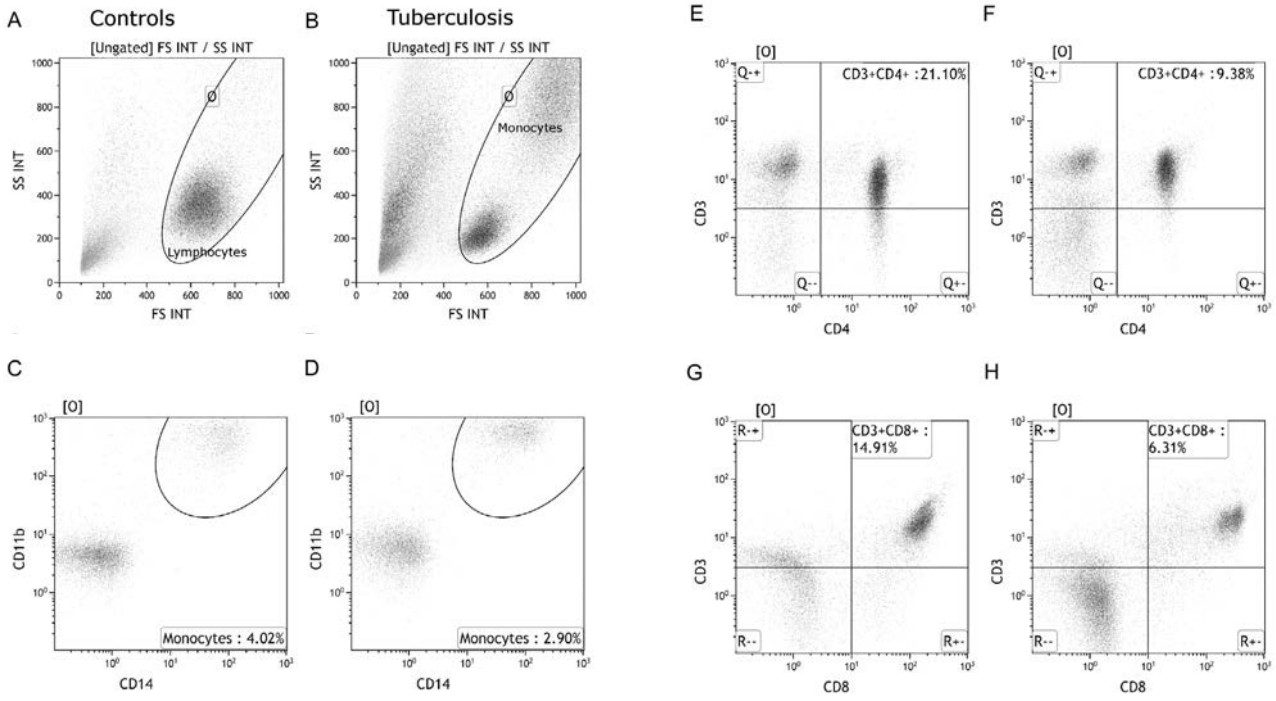

Figure 1. Flow-cytometry images of a healthy control (first column) and patient with tuberculosis (second column) are shown. (A,B,C,D): Monocytes were identified in gated-O cells in the scatter plot of side-scatter (SS) and forward-scatter (FS) with positive CD14 and CD11b. (E,F): $CD3^+CD4^+$ cells were identified in gated-O cells with positive CD3 and CD4. (G,H): $CD3^+CD8^+$ cells were identified in gated-O cells with positive CD3 and CD8.

CA, USA). Flow cytometry analysis was performed using Kaluza software V1.1 (Beckman Coulter, CA, USA). For acquisition and analyses, 2 x 10^5 events were counted in forward and side scatter parameters. Lymphocytes and monocytes were gated in O-area (Fig. 1AB). Setting gates were based on the internal negative controls, which are those cells in the staining sample that do not express the marker. Monocytes were identified based on the positive $CD11b_{PC7}$ and $CD14_{APC\text{-}750}$ expression (Fig. 1CD). CD4 T lymphocytes were identified based on the positive $CD3_{Alexa\ Fluor\ 700}$ and $CD4_{ECD}$ expression (Fig. 1EF). CD8 T lymphocytes were identified based on the positive $CD3_{Alexa\ Fluor\ 700}$ and $CD8_{APC}$ expression (Fig. 1GH). HLA-DR measurements were expressed as percentages of HLA-DR-positive cells and as means of fluorescence intensities (MFI) in relation to the entire cell population, which reflect the HLA-DR density per positive cell (Fig. 2).

Calculation of absolute cell counts

Absolute cell counts were calculated as the

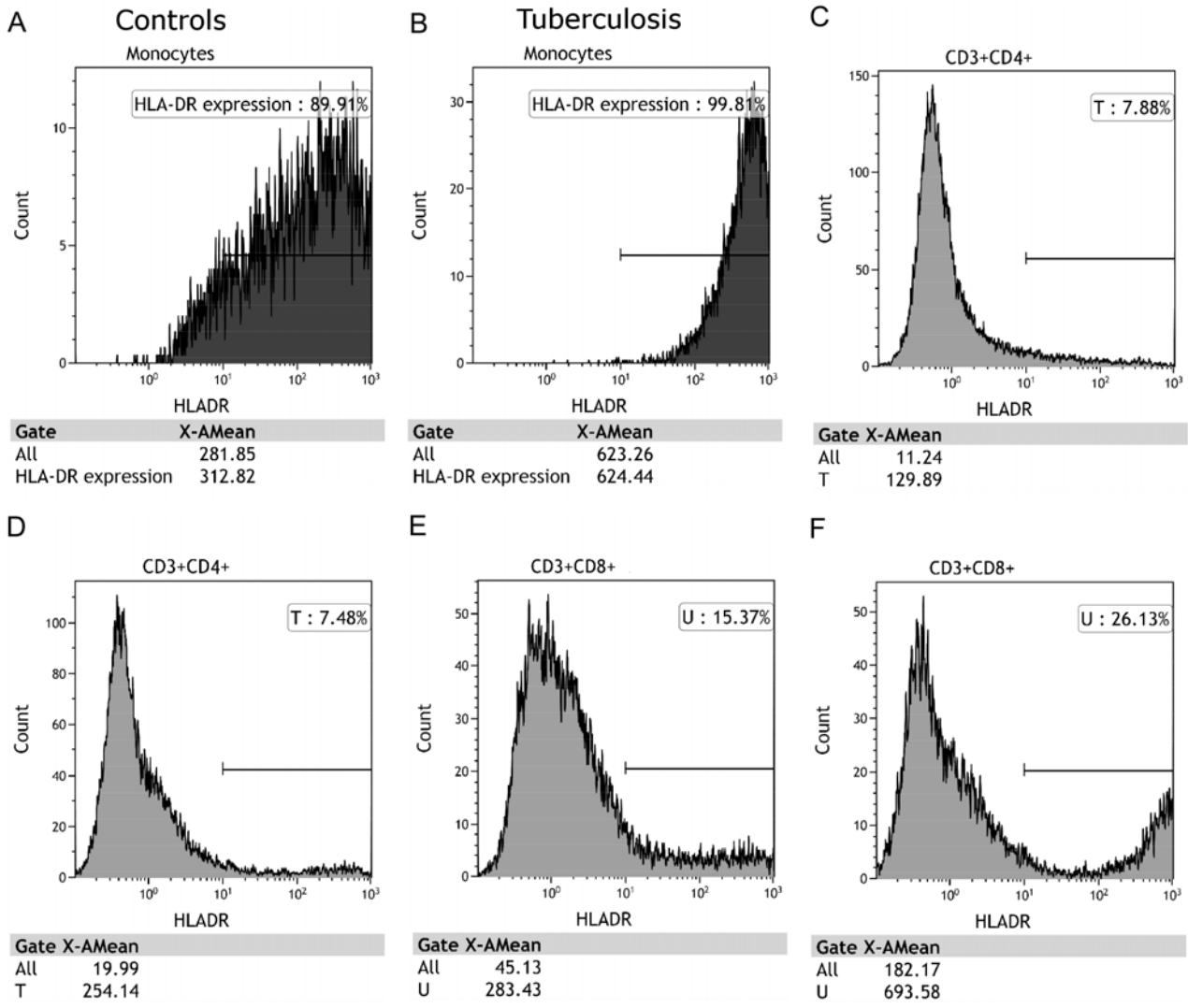

Figure 2. Human leukocyte antigen (HLA)-DR expressions of a healthy control and patient with tuberculosis were represented in the histograms. Positive HLA-DR gating was set by the internal negative population in cells. (A,B): In a healthy control, HLA-DR was expressed in 89.91% of monocytes and the mean of fluorescence intensities (MFI) of HLA-DR was 312.82. (C,D): In a healthy control, HLA-DR was expressed in 7.88% of $CD3^+CD4^+$ cells, and the MFI of HLA-DR was 129.89. (E,F): In a patient of tuberculosis, HLA-DR was expressed in 26.13% of $CD3^+CD8^+$ cells and the MFI of HLA-DR was 693.58.

total PBMC counts multiplied by the percentage of each cells among the PBMCs. Total PBMC counts were obtained from WBC and differential WBC counts that was determined at the hospital's hematology laboratory.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 17.0 for Windows (SPSS Inc., Illinois, USA). Differences in continuous variables were analyzed using the t test. Differences in categorical variables between the two groups were compared using the chi-square test or Fisher's exact test. A p value <0.05 was considered statistically significant.

Results

We found that the CD4/CD8 ratio and percentages of monocytes and CD4 T lymphocytes in controls and in patients with tuberculosis were similar (Table 1). The percentage of CD8 T lymphocytes in patients with tuberculosis was lower than that in controls.

HLA-DR expression in controls and in patients with tuberculosis

Percentages of positive HLA-DR (HLA-DR$^+$) monocytes and CD4 and CD8 T lymphocytes were similar between controls and patients with tuberculosis. However, the HLA-DR MFI in HLA-DR$^+$ monocytes and CD4 T lymphocytes in patients with tuberculosis was lower than that in controls. There was no difference in the HLA-DR MFI in HLA-DR$^+$ CD8 T lymphocytes between controls and patients with tuberculosis. There were no differences in the peripheral blood counts of HLA-DR$^+$ monocytes and CD4 T lymphocytes between controls and patients with tuberculosis. However, the HLA-DR$^+$ CD8 T lymphocyte counts in peripheral circulation in patients with tuberculosis were lower than those in controls due to the lower percentage of CD8 T lymphocytes in PBMCs in patients with tuberculosis.

Discussion

In a study by Barcelos et al., it was found that the HLA-DR$^+$ CD4 T lymphocyte percentage was similar between controls with negative tuberculin skin test and patients with tuberculosis[8]. This finding was similar to the finding in our study. CD4 T lymphocyte HLA-DR expression was decreased in terms of MFI but not in terms of the percentage, and the circulatory CD4 T lymphocyte counts did not change following the *M. tuberculosis* infection. In Fletcher's study, increased HLA-DR$^+$ CD4 T lymphocytes were associated with increased risk of tuberculosis in Bacille Calmette–Guerin vaccinated infants[9]. Thus, CD4 T lymphocyte activation might be affected by tuberculosis. This effect needs to be clarified through more investigations.

CD8 T lymphocytes act as cytotoxic effectors and their main function is to lyse the infected target cells. Thus, decreased CD8 T lymphocyte count or poor CD8 T lymphocyte function is associated with reduced protection against tuberculosis. Our results showed that patients with tuberculosis had lower peripheral activated CD8 T lymphocyte counts compared with controls; this is due to the decreased CD8 T lymphocyte percentage in PBMCs. This result demonstrated that CD8 T lymphocytes play a major role in tuberculosis control. However, in a small study (11 patients with tuberculosis and 9 controls) by Barcelos et al., it was observed that the HLA-DR$^+$ CD8 lymphocyte percentage in patients with tuberculosis was higher than that in controls with negative tuberculin skin test[8], which is in contrast to our observation. More studies are necessary to determine the exact role of activated CD8 T lymphocytes in tuberculosis. In this study CD4/CD8 ratio was similar between controls and patients with tuberculosis, even though the percentage of CD8 T lymphocytes was lower in patients with tuberculo-

Table 1. Clinical characteristics HLA-DR expression in controls and patients with tuberculosis (number, mean ± standard deviation)

	Controls N=27	Tuberculosis N=18
Age, years	61.3 ± 8.4	73.9 ± 14.4
Gender, No. (%)		
Male	19 (70.4)	11 (61.1)
Female	8 (29.6)	7 (38.9)
Co-morbidities, No. (%)		
Chronic obstructive pulmonary disease	0 (0.0)	3 (16.7)
Congestive heart failure	0 (0.0)	1 (5.6)
Hypertension	0 (0.0)	7 (38.9)
Liver cirrhosis	0 (0.0)	4 (22.2)
Chronic renal failure	0 (0.0)	0 (0.0)
Diabetes mellitus	0 (0.0)	6 (33.3)
PBMCs, /μL	2100.2 ± 519.4	1697.5 ± 819.9
Monocytes, %	3.9 ± 1.9	5.3 ± 4.9
CD4 T lymphocytes, %	17.4 ± 8.2	15.1 ± 12.1
CD8 T lymphocytes, %	12.8 ± 4.7	8.0 ± 5.4*
CD4/CD8 ratio	1.9 ± 3.0	2.4 ± 2.0
HLA-DR$^+$ monocytes, /μL	58.6 ± 36.3	63.9 ± 46.2
HLA-DR$^+$ monocytes, %	75.6 ± 21.4	84.0 ± 16.1
HLA-DR MFI in HLA-DR$^+$ monocytes	626.7 ± 193.0	495.1 ± 149.1*
HLA-DR$^+$ CD4 T lymphocytes, /μL	36.8 ± 28.2	24.9 ± 19.5
HLA-DR$^+$ CD4 T lymphocytes, %	10.3 ± 5.5	11.4 ± 5.2
HLA-DR MFI in HLA-DR$^+$ CD4 T lymphocytes	307.4 ± 148.0	172.0 ± 71.5*
HLA-DR$^+$ CD8 T lymphocytes, /μL	68.9 ± 45.0	44.4 ± 40.8*
HLA-DR$^+$ CD8 T lymphocytes, %	27.1 ± 16.0	33.4 ± 12.3
HLA-DR MFI in HLA-DR$^+$ CD8 T lymphocytes	474.8 ± 174.7	435.7 ± 167.3

Abbreviations: HLA=Human leukocyte antigen; PBMCs=Peripheral blood mononuclear cells; MFI=means of fluorescence intensities; HLA-DR$^+$=positive HLA-DR.
*$p<0.05$ compared with the control group using the T test.

sis. The cause of similar CD4/CD8 ratio may be due to relatively lower mean percentage of CD4 T lymphocytes in patients with tuberculosis. This made calculated CD4/CD8 ratio did not show significant difference between controls and patients with tuberculosis.

In this study, the absolute counts of peripheral activated monocytes in patients with tuberculosis were not lower than those in controls, but the MFI in activated monocytes in patients with tuberculosis was lower than that in controls. Similar results have been reported in Sakhno's and Sánchez's studies[10,11]. The authors found a lower percentage of HLA-DR$^+$ monocytes in patients with tuberculosis compared with the controls. However, Barcelos's study showed there was no significant difference in HLA-DR expression in peripheral blood monocytes between 11 patients with tuberculosis and 9 asymp-

tomatic volunteers[8]. A small sample size might be a cause of different results. Our study demonstrated that the percentage of HLA-DR$^+$ monocytes and HLA-DR MFI decreased in the patients with TB, which may have involved in the alteration of function of monocytes caused by the immune exhaustion.

There was one limitation in this study. Some patients with tuberculosis had co-morbidities and healthy controls did not. Although there was no difference between two groups, this might influence immune expression in part.

Conclusions

Patients with tuberculosis had lower activated CD8 T lymphocyte count and lower HLA-DR expression in monocytes and CD4 T lymphocytes compared with controls. Lower HLA-DR MFI in monocytes and CD4 T lymphocyte might be due to immune exhaustion during the long-term inflammatory processes. These findings indicated that tuberculosis might influence parts of the immune system. The exact causal relationship needs more studies to confirm it.

Disclosure of Conflicts of Interest

We confirm that no author has any financial or personal relationships with other organizations that could inappropriately influence this work.

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結核病患周邊血液單核細胞球的人類白血球DR抗原表現

朱建民[1] 高國晉[2,3] 謝孟哲[2,4] 吳黃平[1,2]

[1]基隆長庚醫院 胸腔重症暨睡眠醫學科
[2]長庚大學醫學院
[3]林口長庚醫院 胸腔科
[4]嘉義長庚醫院 胸腔暨重症醫學科

摘 要

身體在對抗結核病時需要免疫反應，然而，結核病患周邊血液單核細胞球的活化情形，目前仍不太清楚，這次研究目的在於探討結核病患單核球及T淋巴球的活化情況。此研究納入27名健康人及18名新診斷的結核病患；利用流式細胞儀測量單核球、CD4 T淋巴球、CD8 T淋巴球的人類白血球DR抗原。研究發現，結核病患CD8 T淋巴球在周邊血液單核細胞球的比例低於對照組，結核病患血液循環CD8 T淋巴球的數目低於對照組；在結核病患中，人類白血球DR抗原陽性的單核球、CD4 T淋巴球，人類白血球DR抗原表現低於對照組。結論是，結核菌感染可能會影響單核球活化，也影響到CD4 T淋巴球、CD8 T淋巴球。