Tuberculosis 95 (2015) 267–274

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

IMMUNOLOGICAL ASPECTS

Mucosal-associated invariant T cells are numerically and functionally deficient in patients with mycobacterial infection and reflect disease activity

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ARTICLE INFO

Article history:
Received 21 October 2014
Accepted 6 March 2015

Keywords:
Mycobacterium tuberculosis
Nontuberculous mycobacteria
Mucosal-associated invariant T cells
Programmed death-1

SUMMARY

Mucosal-associated invariant T (MAIT) cells contribute to protection against certain microorganism infections. The aims of this study were to examine the levels of MAIT cells in pulmonary tuberculosis (TB) and nontuberculous mycobacteria (NTM) lung disease patients, to evaluate the clinical relevance of MAIT cell levels, and to investigate the functions of MAIT cells. Patients with pulmonary TB (n = 35), NTM (n = 29), and healthy controls (n = 75) were enrolled in the study. MAIT cell levels and functions were measured by flow cytometry. Circluating MAIT cell levels were found to be reduced in TB and NTM patients. MAIT cell deficiency reflects a variety of clinical conditions. In particular, MAIT cell numbers were significantly correlated with sputum AFB positivity, extent of disease, hemoglobin levels, lymphocyte counts, CRP and ESR levels. MAIT cells in TB patients failed to produce interferon-γ irrespective of the mode of stimulation, whereas NTM patients displayed a defect in MR1-dependent signaling pathway. Notably, an elevated expression of programmed death-1 was also associated with MAIT cell deficiency in TB. This study shows that MAIT cells are numerically and functionally deficient in TB and NTM patients and these deficiencies could contribute to immune system dysreguation in mycobacterial infection.

1. Introduction

Tuberculosis (TB) remains a serious health problem worldwide, and its burden reached an estimated 8.7 million new cases and 1.4 million deaths for the year 2011 [1]. *Mycobacterium tuberculosis*, a highly infectious pathogen, is spread by the expulsion of the pathogen from patients with pulmonary TB during coughing [2]. It has been known that only 10% of infected individuals develop active tuberculosis and that the remaining 90% of infections are effectively controlled by the hosts [3]. Therefore, host immunity is considered as an important factor in the development of active TB. If appropriate treatment for active TB is not administered, its clinical course is fatal with a 10-year case fatality up to 86% for smear-positive TB [2]. In contrast to *M. tuberculosis*, nontuberculous mycobacteria (NTM) are ubiquitous in the environment and are known to be not transmitted from human to human [4]. NTM lung disease, which is the most common form of NTM infections, is a chronic infectious disease with slow progression [5]. Recently, the incidence of NTM lung disease has been increasing among HIV-negative patients worldwide [6–9].

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http://dx.doi.org/10.1016/j.tube.2015.03.004
1472-9792/

Mucosal-associated invariant T (MAIT) cells are innate-like T lymphocytes that express invariant T cell receptors (TCRs) [10]. Human MAIT cells express an invariant TCR Vα7.2-Jα33 chain paired with a limited repertoire of Vβ chains (i.e., Vβ2 or Vβ13) [11]. Using these unique pairs of TCR chains, MAIT cells recognize bacteria-derived vitamin B2 metabolite antigens presented by the major histocompatibility complex (MHC) class Ib-like related protein MR1 [12,13]. Following Ag recognition, MAIT cells rapidly produce Th1/Th17 cytokines, such as interferon-γ (IFN-γ) and interleukin (IL)-17 in an innate-like manner [14]. Moreover, MAIT cells play important roles in mucosal immunity and gut homeostasis, and contribute to protection against certain mycobacterial and enterobacterial pathogens [15–20].

Previous studies using murine models have shown protective roles of MAIT cells and NKT cells against mycobacterial infections [16,20]. Recently, previous studies have demonstrated numerical and functional deficiencies of MAIT cells in TB patients [20,21]. However, little is known about MAIT cell levels and functions in NTM lung diseases. Moreover, the clinical relevance of MAIT cells in mycobacterial infection has not been determined. The aims of this study were to examine the levels of MAIT cells in pulmonary TB and NTM lung disease patients, to evaluate the relation between MAIT cell levels and clinical parameters, and to investigate the functions of MAIT cells.

2. Materials and methods

2.1. Study subjects

The study cohort was composed of 35 patients with active pulmonary TB, 29 patients with NTM lung disease, and 75 age- and sex-matched healthy controls (HCs). The clinical and laboratory characteristics of the subjects are summarized in Table 1. Pulmonary TB was diagnosed according to the American Thoracic Society diagnostic criteria [2]. TB was confirmed by a positive sputum culture for *M. tuberculosis*. NTM lung disease was defined using the following criteria: (i) the presence of pulmonary symptoms, (ii) the presence of radiographic or high resolution CT scan findings compatible with NTM lung disease, and (iii) positive results from at least two separate expectorated sputum samples for NTM [4]. All HCs met the following criteria: (i) no known exposure to an individual with an active TB infection, (ii) no symptoms of TB infection, (iii) no detectable tuberculin skin test or IFN-γ release assay reactivity, and (iv) no history of autoimmune disease, infectious disease, malignancy, or immunosuppressive therapy [2,22]. The study was approved by the Institutional Review Board of Chonnam National University Hospital (Gwangju, Republic of Korea), and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

2.2. MAbs and flow cytometry

The following monoclonal antibodies (MAbs) and reagents were used in this study: allophycocyanin (APC)-conjugated anti-TCR Vα7.2 (Biolegend, San Diego, CA, USA), APC alexa fluor 750-conjugated anti-CD3 and Pacific Blue-conjugated anti-CD4 (Beckman Coulter, Marseille, France), phycoerythrin (PE)-Cy7-conjugated anti-CD8α, PE-Cy5-conjugated anti-CD161, fluorescein isothiocyanate (FITC)-conjugated anti-TCR γδ, PE-conjugated anti-IFN-γ, PE-conjugated anti-IL-4, PE-conjugated anti-IL-17, and PE-conjugated mouse IgG isotype control (all from Becton Dickinson, San Diego, CA, USA), and PE-conjugated anti-programmed death-1 (PD-1) (eBiosciences, San Diego, CA, USA). Cells were stained with combinations of appropriate mAbs for 20 min at 4 °C. Stained cells were analyzed on a Navios flow cytometer using Kaluza software (Beckman Coulter, Brea, CA, USA).

2.3. Isolation of PBMCs and measurement of MAIT cell numbers

Peripheral venous blood samples were collected in heparin-containing tubes, and peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using Ficoll–Paque Plus solution (Amersham Bioscience, Uppsala, Sweden). MAIT cells were identified phenotypically as CD3+TCRγδ-Vα7.2 + $CD161^{high}$ by flow cytometry as previously described [15,20,23,24]. Total lymphocyte numbers were measured by

Table 1
Clinical and laboratory characteristics of the TB and NTM patients and HCs.

Characteristic	HC	TB	NTM
Total, n	75	35	29
Sex (male/female), n	48/27	23/12	19/10
Age (years), mean ± SD	61.4 ± 11.1	62.7 ± 4.8	64.1 ± 5.6
Previous TB medication, n (%)	ND	6 (17)	13 (45)
Positive sputum AFB smear, n (%)	ND	16 (46)	13 (45)
Cavity on chest radiograph, n (%)	ND	13 (37)	18 (62)
Duration of disease (months), median (range)	ND	1 (1–5)	24 (3–56)
Extent of pulmonary disease, n (%)			
Stage 1 (segmental disease)	ND	1 (3)	3 (11)
Stage 2 (lobar disease)	ND	7 (20)	5 (17)
Stage 3 (bi/trilobar disease)	ND	11 (31)	5 (17)
Stage 4 (bilateral disease)	ND	16 (46)	16 (55)
Mycobacterium species, n (%)			
Mycobacterium tuberculosis	ND	35 (100)	0 (0)
M. intracellulare	ND	0 (0)	22 (76)
M. avium	ND	0 (0)	4 (14)
M. abscessus	ND	0 (0)	2 (7)
M. kansasii	ND	0 (0)	1 (3)
Hemoglobin concn (g/dl), mean ± SD	14.0 ± 1.4	12.0 ± 1.9	12.2 ± 1.8
Leukocyte count (cells/μl), mean ± SD	6197 ± 1593	6588.6 ± 2280.3	6723.1 ± 2290.9
Lymphocyte count (cells/μl), mean ± SD	2174 ± 597.1	1351.4 ± 584.3	1479.0 ± 605.0
Platelet count (× 10^3 cells/μl), mean ± SD	224.3 ± 49.4	263.5 ± 115.7	236.2 ± 78.6
CRP concn (mg/dl), mean ± SD	ND	4.2 ± 6.0	2.1 ± 3.0
ESR (mm/hour), mean ± SD	ND	60.2 ± 31.4	47.5 ± 27.7

AFB = acid-fast bacilli; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; n = number of subjects; ND = not done; SD = standard deviation.