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Circulating mucosal-associated invariant T cell levels and their cytokine levels in healthy adults

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ABSTRACT

Mucosal-associated invariant T (MAIT) cells have been reported to play an antimicrobial role in infectious diseases. However, little is known about age- and gender-related changes in circulating MAIT cell level and function in healthy population. The purposes of this study were to examine the level and cytokine production of circulating MAIT cells and their subsets in healthy adults and to investigate potential relationships between clinical parameters and MAIT cell levels or their subset levels. One hundred thirty-three healthy subjects were enrolled in this study. MAIT cells, their subset, and cytokine levels were measured by flow cytometry. Circulating MAIT cell levels were found to vary widely (0.19% to 21.7%) in the study subjects and to be significantly lower in elderly subjects (age, 61–92 years) than in young subjects (age, 21–40 years) ($p < 0.0005$). No significant difference was found in the circulating MAIT cell levels between male and female subjects. A linear regression analysis revealed that circulating MAIT cell levels declined annually by 3.2% among men and 1.8% among women, respectively. Notably, the proportion of CD4+ MAIT cells increased with age, whereas that of CD8+ MAIT cells decreased with age. In addition, the production of interleukin (IL)-4 by MAIT cells was found to be significantly increased in elderly subjects and the ratio of interferon (IFN)-γ/IL-4 was lower as compared with young subjects, showing a Th1 to Th2 shift in cytokine profile in elderly subjects. Our data suggest that aging is associated with a reduction in circulating MAIT cells, accompanied with alterations in subset composition and cytokine profile.

1. Introduction

Mucosal-associated invariant T (MAIT) cells have recently emerged as an invariant T cell subset, and they play a key role in the protection against several pathogens (Gold et al., 2010; Le Bourhis et al., 2010). MAIT cells express an invariant T cell receptor (TCR) α-chain (Vα7.2-Jα33 in humans and Vα19-Jα33 in mice) paired with a limited set of Vβ chains (Treiner et al., 2003). MAIT cells display a high expression of the C-type lectin CD161 (Walker et al., 2012). Thus, human MAIT cells are phenotypically defined as CD3 + TCRγδ-Vα7.2 + $CD161^{high}$ cells (Dusseaux et al., 2011; Le Bourhis et al., 2010; Martin et al., 2009). In contrast to conventional T cells, which recognize peptide antigens bound to major histocompatibility complex (MHC) molecules, MAIT cells recognize bacterial derived riboflavin (vitamin B2) metabolites presented by the MHC class 1b-like related protein (MR1) (Kjer-Nielsen et al., 2012). Based on the expression of CD4 and CD8, MAIT cells can be subdivided into three subsets: CD4+/CD8− (CD4+), CD4−/CD8+ (CD8+), and CD4/CD8 double-negative (DN) subsets (Leeansyah et al., 2013; Miyazaki et al., 2011). Upon antigen recognition, MAIT cells rapidly produce Th1/Th17 cytokines, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-17, in an innate-like manner (Le Bourhis et al., 2011).

Studies on the levels of circulating MAIT cells and their subsets in a healthy population are very important for identifying changes in MAIT cell levels and their subset compositions in a variety of infectious and autoimmune diseases. Previous studies have demonstrated that the levels of natural killer T (NKT) cells, another invariant T cell subset, are influenced by demographic and environmental factors, such as age, gender, and smoking (Hogan et al., 2011; Jing et al., 2007; Kee et al., 2012; Molling et al., 2005; Montoya et al., 2007; Sandberg et al., 2003). MAIT cells are known to be abundant in peripheral blood,

Abbreviations: APC, allophycocyanin; DN, double-negative; DP, double-positive; FITC, fluorescein isothiocyanate; IFN, interferon; IL, interleukin; IM, ionomycin; MAIT cells, mucosal-associated invariant T cells; MHC, major histocompatibility complex; mAb, monoclonal antibody; NKT cells, natural killer T cells; PBMCs, peripheral blood mononuclear cells; PE-Cy, phycoerythrin-cyanine; PMA, phorbol myristate acetate; TCR, T cell receptor; TNF, tumor necrosis factor.

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Table 1
Demographic and laboratory characteristics of the study subjects.

Characteristics	Total	Male	Female	*P* value
n (%)	133 (100)	69 (51.9)	64 (48.1)	0.9498
Age (years), mean ± SD	50.9 ± 17.6	51.2 ± 17.5	50.6 ± 17.8	0.8392
21–40 (*n*,%)	44 (33.1)	22 (31.9)	22 (34.4)	0.8524
41–60 (*n*,%)	44 (33.1)	23 (33.3)	21 (32.8)	1.0000
61–92 (*n*,%)	45 (33.8)	24 (34.8)	21 (32.8)	0.8558
Hemoglobin (g/dl), mean ± SD	14.0 ± 1.5	14.9 ± 1.2	13.1 ± 1.1	<0.0001
Leukocyte count (cells/μl), mean ± SD	6359 ± 1658.7	6245 ± 1537.7	6485 ± 1841.7	0.3388
Neutrophil count (cells/μl), mean ± SD	3477 ± 1272.8	3314 ± 1112.7	3660 ± 1458.4	0.0918
Lymphocyte count (cells/μl), mean ± SD	2246 ± 651.5	2267 ± 719.2	2222 ± 593.5	0.7488
Monocyte count (cells/μl), mean ± SD	439 ± 193.6	464 ± 214.0	411 ± 180.5	0.0942
Platelet count ($\times 10^3$ cells/μl), mean ± SD	230 ± 54.9	219 ± 51.6	242 ± 58.9	0.0068

n, number of subjects; SD, standard deviation.

representing up to 15% of T lymphocytes (Dusseaux et al., 2011; Martin et al., 2009; Miyazaki et al., 2011). Circulating MAIT cell frequency was reported to be significantly reduced in multiple sclerosis (Miyazaki et al., 2011). However, there are only a few studies evaluating the MAIT cell levels in healthy adults, which have been performed in a small population with an unknown age range and unbalanced gender ratio (Dusseaux et al., 2011; Martin et al., 2009; Miyazaki et al., 2011). Therefore, we considered that a large cohort study including a wide age range and balanced gender ratio was needed to determine the variations in the circulating MAIT cells and their subset levels.

Accordingly, the aim of the present study was to examine MAIT cell levels, their subset levels, and their cytokine levels in the peripheral blood of 133 healthy subjects and to investigate potential relationships between clinical parameters and MAIT cell levels or their subset levels.

2. Subjects and methods

2.1. Study subjects

The study cohort included 133 healthy subjects with ages ranging from 21 to 92 years (48.1% females; mean ± SD age, 50.9 ± 17.6 years). The clinical and laboratory characteristics of the healthy subjects are summarized in Table 1. All subjects were ethnic Korean and none of the subjects had a history of autoimmune diseases, infectious

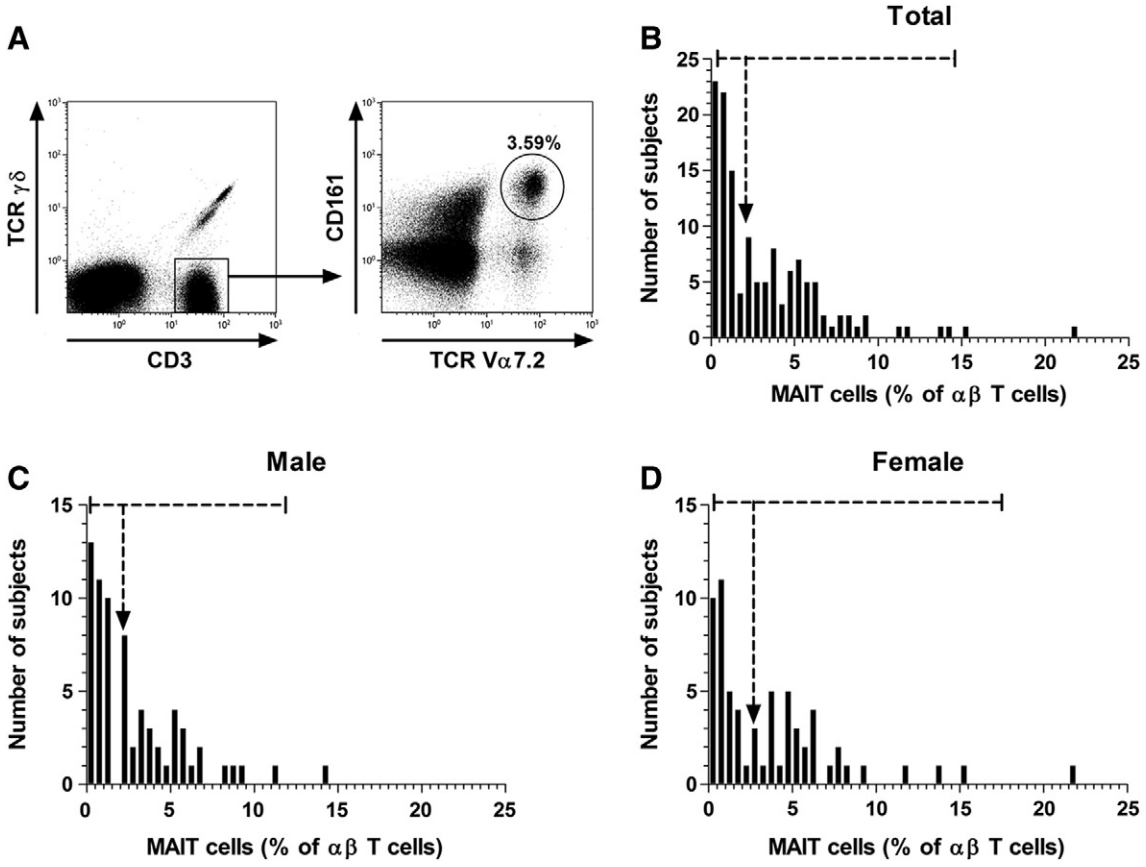

Fig. 1. Distribution of circulating MAIT cell frequencies in the peripheral blood samples of healthy subjects. Freshly isolated PBMCs from healthy subjects were stained with APC Alexa Fluor 750-conjugated anti-CD3, FITC-conjugated anti-TCR γδ, APC-conjugated anti-TCR Vα7.2 and PE-Cy5-conjugated anti-CD161 mAbs. MAIT cells were identified phenotypically as CD3 + TCRγδ- Vα7.2 + CD161 + cells by flow cytometry, and MAIT cell frequencies were expressed as percentages of peripheral blood CD3 + TCRγδ − lymphocytes. (A) Gating strategy and a representative example of flow cytometric analysis of a healthy subject (female, age 23 years). (B–D) Distribution of MAIT cell frequencies in total ($n = 133$), male ($n = 69$), and female ($n = 64$) subjects. Dashed arrows represent medians. Dashed lines indicate the 2.5th to 97.5th percentiles.