Taiwanese Journal of Obstetrics & Gynecology 58 (2019) 117–121

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Less circulating mucosal-associated invariant T cells in patients with cervical cancer

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ARTICLE INFO

Article history:
Accepted 9 March 2018

Keywords:
Cervical cancer
MAIT, mucosal-associated invariant T cell
Immune, Progression-free survival

ABSTRACT

Objective: Mucosal-associated invariant T cells (MAITs) are important for immune defense against infectious pathogens and regulation of various inflammatory diseases. However, their roles in cancer are rarely reported. Since cervical cancer is one of the diseases involving mucosal tissue, we try to investigate the association between circulating MAITs and cervical cancer.

Materials and methods: Blood samples were obtained from patients with cervical cancer (n = 47) and healthy individuals (n = 39). We determined phenotypic MAITs in peripheral blood mononuclear cells (PBMCs) and evaluated the percentage of MAITs in CD3+ cells by flow cytometry. The percentage of MAITs was stratified according to Federation of Gynecology and Obstetrics (FIGO) staging system in patients with cervical cancer. Progression-free survival (PFS) with respect to the amount of MAITs was also analyzed.

Results: The percentage of circulating MAITs in patients with cervical cancer was significantly lower than in healthy group (0.987% vs. 4.008%, $p < 0.0001$). In subgroup analysis, though not statistically significant, it showed a trend of lower percentage of circulating MAITs in cervical cancer patients with FIGO stage II-IV disease than in patients with FIGO stage I disease (0.4045% vs. 1.098%, $p = 0.11$). A trend of poor PFS in patients with lower circulating MAITs was also noted.

Conclusion: MAITs play a crucial role in cancer immunity. The decrease of MAITs in peripheral blood is related to cervical cancer. There is a trend of lower percentage of MAITs in advanced stages and lower percentage of MAITs towards poor PFS in patients with cervical cancer.

Introduction

Over the last few years, mucosal-associated invariant T cells (MAITs), an evolutionarily conserved T lymphocyte subset was identified and characterized. They express invariant T cell antigen receptor (TCR) α chains containing Vα7.2 and Jα33 [1]. MAITs recognize antigens presented by an invariant major histocompatibility complex-related 1 (MR1) molecule [2]. These cells usually reside in the lamina propria of bowel mucosa and act as a defender against infection. Although MAITs are sparse in mouse, they are relatively abundant in human and account for 1–10% of T cells in peripheral blood or in liver mucosal tissues [2]. Until recently, little is known about the detailed characterization of MAITs-MR1 because of lack of method for the identification and isolation of these cells. MAITs were mostly described as innate defender of infection for recognizing the vitamin B metabolites, which are generated by many bacteria [3].

For ovarian cancer, significant differences in the distributions of progression-free survival (PFS) and overall survival (OS) according to the presence or absence of intra-tumoral T cells ($P < 0.001$ for both comparisons) were seen in patients with complete response after debulking surgery and chemotherapy. The presence of intra-tumoral T cells independently correlated with delayed recurrence and delayed death. The weight of presence of tumor infiltrating T cells (TILs) is even higher than debulking surgery [4]. It is interesting to know if MAITs play a crucial role as TILs or any correlation

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https://doi.org/10.1016/j.tjog.2018.11.022

between TILs and MAITs. Circulating MAITs also play a role in autoimmune disease such as inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE) [4,5]. MAITs are activated by cytokines, which result in increased recruitment of themselves and increased more cytokine secretion. Their activated status correlates with presence of disease, and reflects disease activity [4,5]. For colorectal cancer (CRC), increased tumor infiltration by MAITs correlates with advanced stage [6] and poor survival [7], on the other side, decreased circulating MAITs correlates with advanced disease [6]. However, the roles of MAITs in tumor immunology and their characteristic in gynecologic cancer are still not clear and needed to be further investigated. Our study is to explore the possible relationship of circulating MAITs in cervical cancer and their potential clinical significance in cervical cancer.

Materials and methods

Human samples

Forty-seven primary cervical cancer patients who underwent surgery were enrolled in this study, from September 2011 to March 2016 in MacKay memorial hospital, Taipei, Taiwan. We also enrolled healthy individuals (n = 39) that exclude premalignant disease or other gynecologic malignancy. Blood samples were obtained from these two groups for analysis. Cervical cancer was diagnosed by biopsy and proved by pathology after surgery. Cancer stages, histology types including squamous cell carcinoma (SCC) (n = 32), adenocarcinoma (n = 13), and adenosquamous cell carcinoma (n = 2) were documented. The detailed FIGO stage (2009) and histology types of cervical cancer are listed in supplementary table. PFS was defined as the time (in days) between surgery and any disease-recurrent related event, i.e., relapse or death. All participants gave written informed consent to this study, according to ethical guidelines and under the approval of Institutional Review Board (No. 16MMHIS139).

Phenotype of MAITs and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were purified from heparinized blood by centrifugation according to standard protocol. Isolated PBMCs were washed and stained with fluorescent antibodies as following: fluorescein isothiocyanate (FITC) anti-human CD3 antibody (BioLegend, 317306), PE γδ TCR monoclonal antibody (eBioscience, MHGD04), peridinin chlorophyll (PerCP)/cyanine 5.5 (Cy5.5) anti-human TCR Vα7.2 antibody (BioLegend, 351710) and APC CD161 antibody (eBioscience, 48-0048-42). Phenotype of MAITs was defined as CD3+TCRγδ-Vα7.2 + CD161++ (Fig. 1). Hematologic components were analyzed through flow cytometry (BD FACSCalibur). We then measured the percentage of MAITs in CD3+ T cells. The frequency of MAITs was calculated as follow: percentage of MAITs = (number of CD3+TCRγδ-Vα7.2 + CD161++/number of CD3+).

Statistics

Statistical analyses for clinical data were performed with unpaired Student's *t*-tests. The Kaplan–Meier method was used for calculation of progression free survival and the log-rank test for

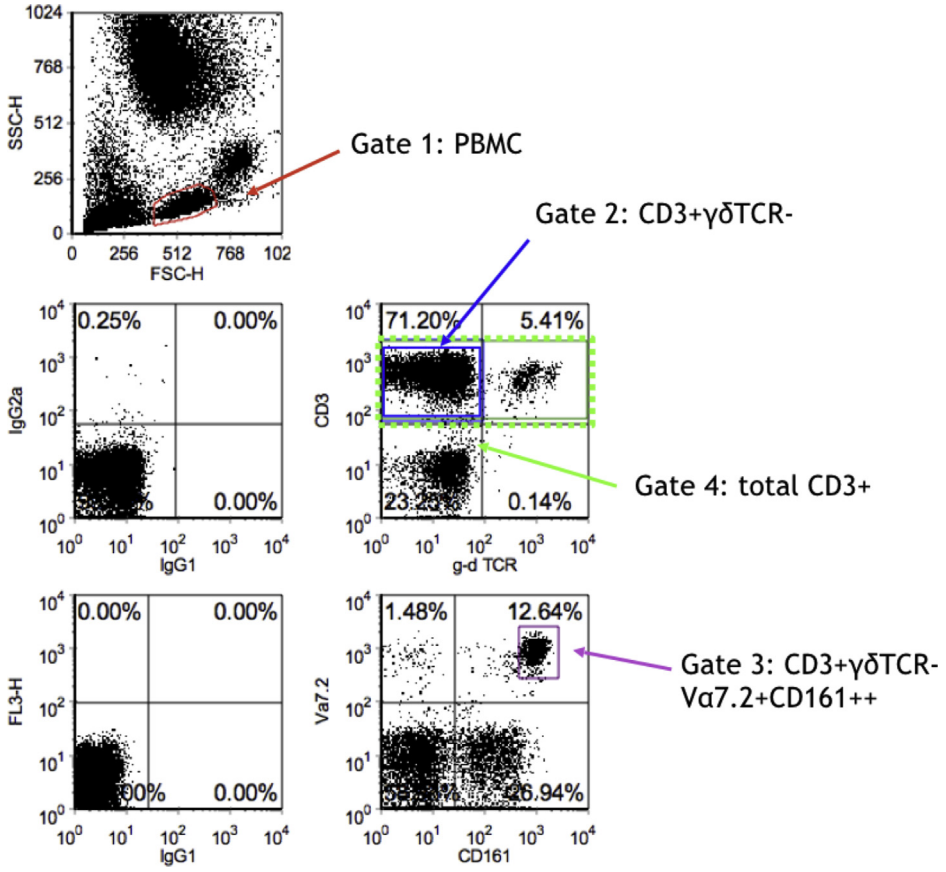

Fig. 1. Flow cytometry analysis of MAITs. PBMCs were isolated from blood samples (Gate 1) and stained with fluorescent antibodies against CD3, CD161, TCRγδ, and TCRVα7.2. The frequency of CD3+TCRγδ-Vα7.2 + CD161 + MAITs was characterized by flow cytometry. The percentage of MAITs was counted by events in gate 3 divided by events in gate 4, representing MAITs among CD3+ cells.

comparison of survival curves. In both cases, a value of $p < 0.05$ was considered significant.

Results

A total of forty-seven newly diagnosed cervical cancer patients and thirty-nine healthy controls were enrolled in this study. The frequency of circulating CD3+TCRγδ-Vα7.2 + CD161++ cells in total CD3+ lymphocytes in individual subjects was determined by flow cytometry, and the percentage of MAITs of CD3+ cells was calculated. The results revealed that the percentage of circulating MAITs was significantly lower in cervical cancer patients than in healthy controls (0.987 ± 0.19% vs. 4.008 ± 0.59%, $p < 0.0001$) (Fig. 2). The age range of patients with cervical cancer was from 36 to 77 years old and the age range of healthy individuals was from 29 to 46 years old. The average ages between these two groups were indeed different, that healthy individuals were younger than patients with cervical cancer (40.05 ± 0.69 vs. 53.11 ± 1.6 years old). Since the age of subjects may affect the percentage of MAITs between the two groups, the age was adjusted to below 45 years old among patients with cervical cancer, that the average age between the two groups was not different (40.05 ± 0.69 vs. 41.13 ± 1.00 years old). Then, the percentage of MAITs among CD3+ T cells was compared between healthy individuals and cervical cancer patients after age-adjustment. There was still significantly lower percentage of circulating MAITs in patients with cervical cancer after age-adjustment than in healthy individuals (1.869 ± 0.44% vs. 4.008 ± 0.59%, $p = 0.0319$) (Fig. 3).

In order to define if there is a relationship between lower percentage of MAITs and advanced cancer stage, we analyzed percentage of MATIs stratified according to FIGO stages. The percentage of MAITs in cervical cancer patients with FIGO stage II-IV disease (0.405 ± 0.22%, $n = 12$) is lower compared to FIGO stage I disease (1.098 ± 0.23%, $n = 35$), $p = 0.11$ (Fig. 4). We also evaluated the difference according to AJCC (TNM) stage, and it revealed the same results. Although there is no statistically significant difference, we discover a trend toward lower percentage of circulating MAITs in cervical cancer patients with advanced stage.

Besides, we tried to understand if there is a relationship between the percentage of circulating MAITs and PFS, regardless of FIGO stages. Kaplan–Meier analysis of PFS between two groups of patients stratified according to percentage of MAITs (≥0.5% or <0.5%, ≥1% or <1%, ≥2% or <2%) was performed. Similar results were observed in MAITs according to different percentages. For instance, there was a trend toward better PFS (Fig. 5) in the patient group with higher circulating MAITs (>1%) compared to lower circulating MAITs (<1%), $p = 0.55$. Although the difference was not statistically significant between the two groups stratified by the percentages of MAITs, the trend is obvious that lower percentage of MAITs is related to poorer PFS.

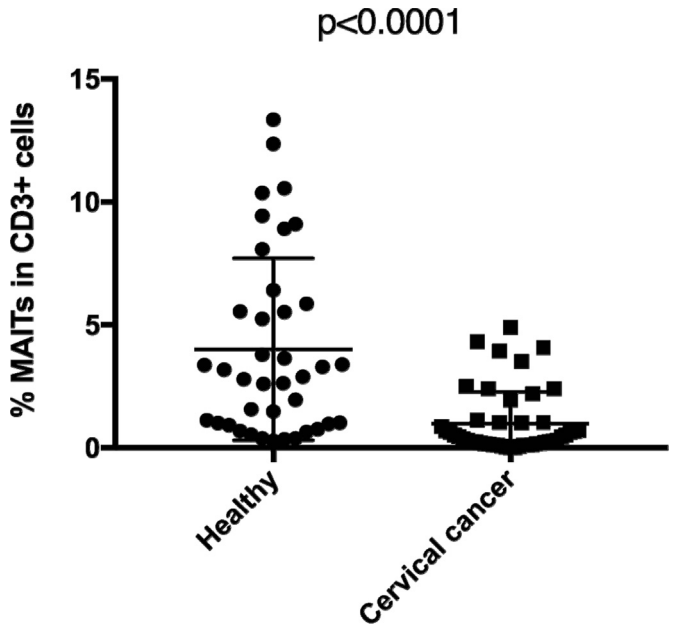

Fig. 2. Analysis of percentage of MAITs in healthy controls and cervical cancer patients. Percentage of MAITs among CD3+ cells from isolated PBMCs in healthy controls and cervical cancer patients was measured. Significantly lower percentage of MAITs among CD3+ cells was noted in cervical cancer patients than in healthy controls (0.987% v.s. 4.008%, $p < 0.0001$).

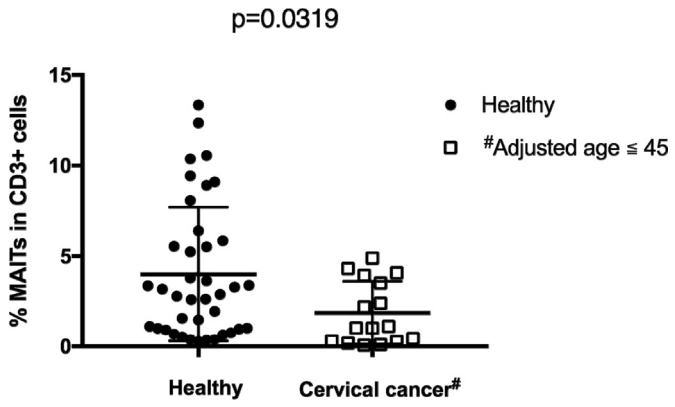

Fig. 3. Analysis of percentage of MAITs in healthy controls and cervical cancer patients with adjusted age. Percentage of MAITs in healthy controls and cervical cancer patients after age-adjustment was measured. The age of individuals between the two groups was not different. Still, significantly lower percentage of MAITs in cervical cancer patients after age-adjustment than in healthy controls was noted (1.869 ± 0.44% v.s. 4.008 ± 0.59%, $p = 0.0319$).

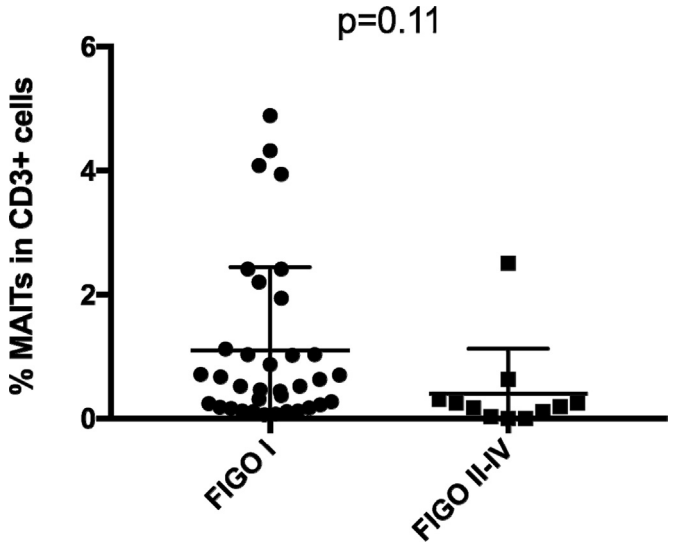

Fig. 4. Association between percentage of MAITs and stages in cervical cancer patients. Percentage of MAITs was measured according different stages in cervical cancer patients. There was a trend of lower percentage of MAITs in more advanced stage (FIGO II-IV) than in early stage (FIGO I) (0.4045% v.s. 1.098%, $p = 0.11$). The difference was not statistically significant between the two groups.

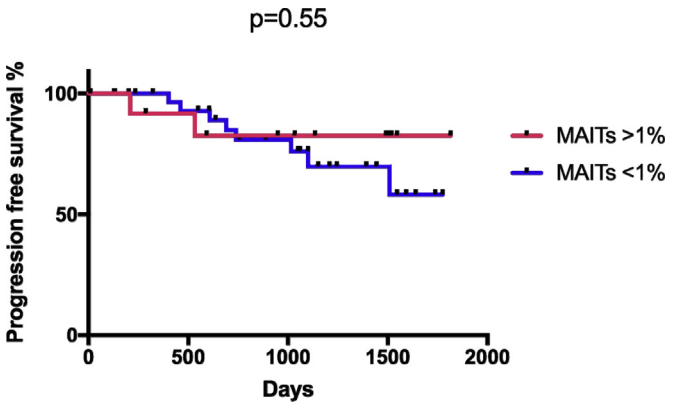

Fig. 5. Kaplan–Meier analysis of progression free survival between two groups of patients stratified according to percentage of MAITs (>1% or <1%). There was a trend toward poorer PFS in the patient group with lower circulating MAITs (<1%) compared to higher circulating MAITs (>1%). The difference was not statistically significant between the two groups.

Discussion

Little was known about MAITs, however until recently, several advances of their characteristics and functions in immunology field and in clinical practice were confirmed. Previous studies have suggested that CD3+TCRγδ-Vα7.2 + CD161++ cells can be considered as MAITs [8–10]. MAITs activation can result from either TCR-dependent signaling (triggered by ligand presented on MR1 by antigen-presenting cells) or from TCR-independent cytokine signaling [11,12]. Like other 'innate-like' lymphocytes including invariant natural killer T cells and natural killer cells, MAITs respond rapidly to activation by producing Th1/Th17 cytokines, such as IFN-γ and IL-17 [11,13]. Increasing evidences suggest that MAITs play a protective role in anti-bacterial immunity at mucosal interfaces, but the role in fungal and viral infection has not yet been proofed [14]. MAITs may become enriched at the site of infection and possibly contribute to the early anti-bacterial immune response, that circulating MAITs become depleted and the remaining population is activated, exhausted and dysfunctional [14]. Therefore, we are inspired by that MAITs become deprived in peripheral blood after responding to stimuli. MAITs also play a role in autoimmune and immune-mediated diseases including SLE, rheumatoid arthritis (RhA), multiple sclerosis (MS), IBD and airway diseases [15]. MAIT deficiencies are frequently observed in peripheral blood, and at sites of disease such as the airways in asthma [15]. Reduced frequency of human MAITs in peripheral blood in patients with both SLE and RhA, which are correlated with disease activity scores in both conditions [16]. MAITs have been shown to be decreased in peripheral blood of MS patients, particularly during disease relapse, compared with disease remission [17]. MAITs are also activated in IBD, which result in increased recruitment toward the inflamed tissue, and lead to deficiency of MAITs in peripheral blood in both Crohn's disease and ulcerative colitis [4,18]. These findings suggest possible recruitment of MAITs to sites of disease, and depletion of peripheral circulation MAITs is related to disease severity.

It attracts our attention that CD3+ tumor-infiltrating T cells detected within tumor-cell islets (intra-tumoral T cells) are independently correlated with delayed recurrence and delayed death in advanced ovarian cancer patients [19]. The roles of circulating and tumor-infiltrating MAITs in cervical cancer are still unclear. It is believed that circulating MAITs level was significantly reduced in patients with mucosal-associated cancers, such as gastric, colon and lung cancers [20]. The percentages of circulating memory CD8+ MAITs were significantly reduced while tumor infiltrating MAITs were increased, especially in patients with advanced CRC [6], also, the proportion of MAITs among total CD3+ T cells showed significant increased frequency of MAITs within the tumor by compared to paired samples with healthy tissues [7].

In our study, analysis of MAITs in PBMCs showed a promising result, which the percentage of circulating MAITs in CD3+ T cells is significantly lower in cervical cancer patients than in healthy controls. In our study, the percentage of circulatory MAITs is about 4% in healthy individuals, that is comparable to 1–10% in human from previous study [2], however the percentage of MAITs in patients with cervical cancer is less than 1% (0.987%). In a previous population-based study, the amount of MAITs reaches maximal levels in the third and fourth decenniums and the values then dramatically decline; both absolutely and as a percentage among CD3+ T cells, than subjects in fertile age [10]. The age range of healthy individuals was from 29 to 46 years in our study that they were indeed younger. In order to eliminate the influence by age, we adjusted age range among patients with cervical cancer to below 45 years old that the age range was from 36 to 45 years old, therefore the average ages between these two groups were not different. Percentage of MAITs among CD3+ T cells was still significantly lower in patients with cervical cancer after age adjustment. We can conclude that in the similar age group, the percentage of MAITs is lower in patients with cervical cancer than in healthy individuals.

Cervical cancer is a mucosal-associated cancer like CRC, although not yet studied, we believe that MAITs are recruited into tumor tissue and result in decreased circulatory level just like CRC. Also, the percentage of MAITs was lower in cervical cancer patients with FIGO stage II-IV disease (0.405%) compared to patients with FIGO stage I disease (1.098%), despite the difference was not statistically significant. We assumed the following reasons may affect the result so the difference was not significant. First, the patient number in two groups showed discrepancy (FIGO I, n = 35; FIGO II-IV, n = 12), and second, the patient number was too small in FIGO II-IV group. Besides, we also analyzed percentage of MAITs in different histology types of cervical cancer, such as SCC, adenocarcinoma, adenosquamous; and there was no significantly statistical difference. The PFS in patients with cervical cancer stratified according to different percentages of MAITs (0.5%, 1%, and 2%) showed no significantly statistical difference. However, it showed a trend of poor PFS towards lower percentage of circulating MAITs. Since there were few events of recurrence and only two deaths, we did not analyze overall survival in this study. The limitation of the study is the small number in subgroup analysis, as for FIGO stage and histology type, and we need more time for long-term follow up for survival benefits regarding overall survival.

In summary, our datas indicate there is significantly lower frequency of circulating MAITs in patients with cervical cancer than in healthy individuals. MAITs may participate in the immune surveillance of cervical cancer and probably play an important role as TILs which recruit to tumor site and result in decrease of peripheral circulating MAITs. We anticipate that tumor infiltrating MAITs in cervical cancer will be further investigated and its correlation with survival benefit can be identified. Our findings may provide new insights in the role of MAITs in regulating disease progression of cervical cancer. In the future, more studies are needed to explore the mechanistic roles of MAITs in cancer microenvironment and strategies involving MAITs in cancer treatment.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgement

The authors express sincere gratitude to all the colleagues who participated this study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.tjog.2018.11.022.

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