Activation and Impaired Tumor Necrosis Factor- α Production of Circulating Mucosal-Associated Invariant T Cells in Patients with Trauma

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Keywords

Mucosal-associated invariant T cells · Proinflammatory cytokine · Trauma · Tumor necrosis factor- α

Abstract

Mucosal-associated invariant T (MAIT) cells rapidly produce proinflammatory cytokines in an innate-like manner and play an important role in controlling the host immune response. This study examined the function of MAIT cells in trauma patients. The expression of cytokines in peripheral blood MAIT cells was measured by flow cytometry. MAIT cells in trauma patients displayed impaired tumor necrosis factor (TNF)- α production, together with elevated CD69 expression. The expression of CD69 was negatively correlated with MAIT cell frequency. These patients had higher plasma levels of interleukin (IL)-12 and IL-18. In particular, CD69 expression of MAIT cells was increased by stimulation with IL-18 in synergy with other proinflammatory cytokines or plasma of trauma patients. The production of TNF- α by MAIT cells was characterized by an initial burst and rapid decline, in contrast to delayed and sustained production of interferon (IFN)- γ . Activated MAIT cells showed a functional defect in the production of TNF- α upon restimulation. This study demonstrates that circulating MAIT cells are activated and

functionally impaired in TNF- α production in patients with trauma. The activation and dysfunction of MAIT cells was mediated by proinflammatory cytokines. These findings provide important information underlying the innate immune response of patients with trauma.

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Introduction

Trauma represents one of the major causes of death and disability worldwide. According to the Global Burden of Disease and Injury study, 973 million people sustained traumatic injuries and 4.8 million people died from their injuries in 2013 [1]. Severe traumatic injury evokes an acute, non-specific, systemic inflammatory response syndrome (SIRS). It is associated with poor outcome after trauma, paradoxically reducing the body's ability to fight infection [2, 3], although the purpose of the inflammatory response is to protect the body against infection. After tissue damage, the injured tissues or activated immune cells release endogenous factors such as damage-associated

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molecular patterns (DAMPs) or alarmins [2, 4], which are potent activators of innate immune cells (e.g., neutrophils and monocytes) and complement (e.g., C3a and C5a), via cell-surface DAMP receptors [2, 5–7]. Activation of inflammatory and complement systems triggers the production of cytokines and chemokines, thereby generating SIRS [8]. The degree of immune system activation is related to the levels of such cytokines, and is associated with mortality, multiple organ dysfunction syndrome (MODS), and sepsis [9]. Therefore, a better understanding of the activation of innate immune response by trauma may lead to novel strategies for the improvement of patient outcome after trauma [2].

Mucosal-associated invariant T (MAIT) cells are innate lymphocytes that express a conserved invariant T cell receptor (TCR) V α 7.2-J α 33 chain paired with a limited set of V β chains [10]. MAIT cells are mainly found in the peripheral blood and other tissues like liver, lung, and mucosa [11]. Using distinct pairs of TCR chains, MAIT cells recognize bacteria-derived riboflavin (vitamin B₂) metabolites presented by the MHC class 1b-like related protein (MR1) [10, 12]. Upon MR1-dependent recognition of antigens, MAIT cells are activated to rapidly release Th1/Th17 proinflammatory cytokines, including interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and interleukin (IL)-17, and cytotoxic molecules like granzyme and perforin to kill infected host cells [13]. MAIT cells are also activated in an MR1-independent manner, depending on IL-18 expression in synergy with other inflammatory cytokines. Previous reports show that MAIT cells express increased levels of IL-18R α and IL-12R [14], and are activated by IFN- α , IL-15, and IL-12+IL-18 in the absence of exogenous antigens [15–17].

Our previous study demonstrated that MAIT cells are deficient in multiple trauma patients and their deficiency is associated with the severity of trauma [18]. MAIT cells play an important role in the innate immune response to traumatic injury. However, little is known about the functional activation of MAIT cells in trauma. Accordingly, the goal of this study was to examine the production capacity of cytokines in peripheral blood MAIT cells of trauma patients.

Patients and Methods

Patients

The study cohort was composed of 16 patients with trauma (6 females and 10 males; mean age $\pm$ standard deviation [SD] 61.4 $\pm$ 15.2 years) who visited the Chonnam National University Hospi-

Table 1. Clinical and laboratory characteristics of the 16 trauma patients

Parameters	Findings
Sex (male/female)	10/6
Age, years	61.4 $\pm$ 15.2
<i>Clinical variables</i>	
APACHE II score	11.1 $\pm$ 9.9
SAPS II score	29.5 $\pm$ 16.4
ISS score	18.5 $\pm$ 10.6
ISS categories,	
Mild (<9)	2 (12.5)
Moderate (9–15)	4 (25)
Severe (>15)	10 (62.5)
Mortality	3 (18.75)
<i>Laboratory variables</i>	
Leukocytes, cells/ μ L	9,019 $\pm$ 2,828
Lymphocytes, cells/ μ L	1,127 $\pm$ 474
Neutrophils, cells/ μ L	7,208 $\pm$ 2,635
Monocytes, cells/ μ L	639 $\pm$ 313
Hemoglobin, g/dL	10.9 $\pm$ 1.7
Platelets, $\times 10^3$ / μ L	140 $\pm$ 41
Bilirubin, mg/dL	0.9 $\pm$ 0.4
BUN, mg/dL	18.8 $\pm$ 15.9
Creatinine, mg/dL	1.4 $\pm$ 1.9
CRP, mg/dL	4.8 $\pm$ 5.5
PaO ₂ , mm Hg	100.8 $\pm$ 38.4
Lactate, mmol/L	2.9 $\pm$ 2.0
Bicarbonate, mmol/L	23.0 $\pm$ 5.1
Prothrombin time (INR)	1.30 $\pm$ 0.32
MAP, mm Hg	78 $\pm$ 22
Heart rate, beat/min	102 $\pm$ 25
Body temperature, $^{\circ}$ C	37.1 $\pm$ 0.5

Data are presented as *n* (%) or the mean $\pm$ SD. APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score; ISS, Injury Severity Score; BUN, blood urea nitrogen; CRP, C-reactive protein; PaO₂, partial pressure of oxygen in arterial blood; INR, international normalized ratio; MAP, mean arterial pressure.

tal Regional Trauma Center after the trauma and 16 non-injured healthy controls (HCs; 7 females and 9 males; mean age $\pm$ SD 52.4 $\pm$ 5.5 years). All blood samples were usually obtained from the patients within 12–36 h after the traumatic injury. The clinical and laboratory characteristics of the patients are summarized in Table 1.

Monoclonal Antibodies and Flow Cytometry

The following monoclonal antibodies (mAbs) and reagents were used in this study: allophycocyanin (APC)-Cy7-conjugated anti-CD3 (SK7), phycoerythrin (PE)-Cy5-conjugated anti-CD161 (DX12) and fluorescein isothiocyanate (FITC)-conjugated anti-TCR $\gamma\delta$ (11F2), FITC-conjugated anti-CD3 (HIT3a), FITC-conjugated anti-IFN- γ (B27), FITC-conjugated annexin V, PE-conju-

gated anti-CD3(HIT3a), PE-conjugated anti-IL-17 (SCPL1362), PE-Cy7-conjugated anti-TNF- α (Mab11), PE-conjugated anti-CD69 (FN50), FITC-conjugated mouse IgG isotype (X40), PE-conjugated mouse IgG isotype (X40) and PE-Cy7-conjugated mouse IgG isotype (MOPC-21) control (all obtained from Becton Dickinson, San Diego, CA, USA), PE-conjugated anti-programmed death-1 (anti-PD-1; MIH4; eBioscience, San Diego, CA, USA) and APC-conjugated anti-TCR Va7.2 (3C10; BioLegend, San Diego, CA, USA). Cells were stained with combinations of appropriate mAb for 20 min at 4°C. The stained cells were analyzed on a Navios flow cytometer using Kaluza software (version 1.5a; Beckman Coulter, Brea, CA, USA). MAIT cells were identified phenotypically as CD3+TCR $\gamma\delta$ -Va7.2+CD161^{high} cells using flow cytometry as previously described [19, 20].

Functional MAIT Cell Assay

The expression of IFN- γ , IL-17, and TNF- α in MAIT cells was detected by intracellular cytokine flow cytometry as previously described [20]. To determine the CD69 expression in MAIT cells after stimulation with cytokine cocktail or plasma from trauma patients, freshly isolated PBMCs (1×10^6 /well) were stimulated with a proinflammatory cytokine cocktail consisting of IL-6 (50 ng/mL; PeproTech), IL-8 (10 ng/mL; PeproTech), IL-12 (50 ng/mL; Miltenyi Biotec, Bergisch Gladbach, Germany), IL-18 (50 ng/mL; Medical and Biological Laboratories, Woburn, MA, USA), and TNF- α (5 ng/mL; PeproTech) for 24 h, or stimulated with 300 μ L of trauma patient plasma for 3 days. Cells were stained with FITC-conjugated anti-CD3, APC-conjugated anti-TCR Va7.2, PE-conjugated anti-CD69, and PE-Cy5-conjugated anti CD161 mAbs for 20 min at 4°C. CD69+ MAIT cells were determined by flow cytometry. Blocking antibodies for cytokines included anti-IL-18 (5 μ g/mL; R&D Systems) and anti-IL-6 (5 μ g/mL), anti-IL-8 (5 μ g/mL), anti-IL-12 (5 μ g/mL), and anti-TNF- α (5 μ g/mL; all from BD Biosciences).

To determine the production of IFN- γ and TNF- α by MAIT cells after restimulation, freshly isolated PBMCs were stimulated with Dynabeads Human T-Activator CD3/CD28 (Life Technologies) and cytokine cocktail consisting of IL-6 (50 ng/mL), IL-8 (10 ng/mL), IL-12 (50 ng/mL), IL-18 (50 ng/mL), and TNF- α (5 ng/mL) for 16 h. The cells were washed to remove the activating factors and cultured with IL-2 (100 U/mL; BD Pharmingen) to support cell survival for 1 day. Later, cells were restimulated for 16 h with the same stimulators. The production of IFN- γ and TNF- α by MAIT cells was determined by intracellular flow cytometry as described above.

Statistical Analysis

The expression levels of IFN- γ , IL-17, TNF- α , CD69, annexin V, and PD-1 in MAIT cells between HCs and patients were compared by analysis of covariance after adjusting for age and sex using Bonferroni correction for multiple comparisons. The expression of IFN- γ , TNF- α , and CD69 between unstimulated and stimulated or between stimulated and restimulated MAIT cells was compared using a paired *t* test. The Mann-Whitney U test was used to compare plasma levels of cytokines in trauma patients versus age- and sex-matched HCs. Spearman's correlation analysis was used to examine the relationships between MAIT cell percentages and CD69+ MAIT cells, annexin V+ MAIT cells, or PD-1+ MAIT cells. *p* values <0.05 were considered statistically significant. Statistical analysis and graphic works were performed using SPSS version

18.0 software (SPSS, Chicago, IL, USA) and GraphPad Prism version 5.03 software (GraphPad Software, San Diego, CA, USA), respectively.

Results

Impaired TNF- α Production of Circulating MAIT Cells in Trauma Patients

To examine the expression of inflammatory cytokines in MAIT cells, we incubated PBMCs derived from 16 trauma patients and 16 HCs for 4 h in the presence of phorbol myristate acetate (PMA) and ionomycin (IM). The expression of IFN- γ , IL-17A, and TNF- α in the MAIT cell populations was determined at the single-cell level by intracellular flow cytometry (Fig. 1a). Percentages of TNF- α -expressing MAIT cells were found to be significantly lower in trauma patients as compared with HCs (median 38.0 vs. 56.2%, *p* < 0.01). However, IFN- γ + and IL-17A+ MAIT cell levels were comparable between the patients and HCs (Fig. 1b).

Activation of MAIT Cells in Trauma Patients

Our previous study reported deficiencies of MAIT cell numbers in patients with trauma [18]. To determine whether circulating MAIT cell deficiency is associated with activation-induced cell death, we investigated the activation and apoptosis, indicated by CD69 upregulation and annexin V staining, respectively, in circulating MAIT cells. CD69+ and annexin V+ MAIT cells were examined by flow cytometry. Percentages of CD69+ MAIT cells were found to be significantly higher in trauma patients than in HCs (median 25.5 vs. 6.1%; *p* < 0.0001, Fig. 2a, b). In particular, the CD69 expression of MAIT cells was inversely correlated with the frequency of MAIT cells in trauma patients (γ = -0.562, *p* < 0.05; Fig. 2c). However, no significant difference was observed between the annexin V+ MAIT cell levels in patients and HCs (*p* = 0.25; Fig. 2d, e). Furthermore, no significant correlation was observed between MAIT cell percentages and annexin V+ MAIT cells (Fig. 2f).

To determine whether impaired TNF- α production of MAIT cells was related to PD-1, we evaluated the expression of PD-1 in MAIT cells from 16 patients and 16 HCs. The expression of PD-1 in the MAIT cell populations was examined at the single-cell level by flow cytometry (Fig. 2g). Expression levels of PD-1 in MAIT cells were comparable between patients and HCs (*p* = 0.64; Fig. 2h). No significant correlation was observed between MAIT cell percentages and PD-1+ MAIT cells (Fig. 2i).

Fig. 1. Decreased expression of TNF- α in MAIT cells of trauma patients. Freshly isolated PBMCs (1×10^6 /well) were incubated for 4 h in the presence of PMA (100 ng/mL) and IM (1 μ M). **a** Representative IFN- γ , IL-17, and TNF- α expressions in the MAIT cell population were determined by intracellular flow cytometry after stimulation with PMA and IM. **b** Data related to IFN- γ +, IL-17+, and TNF- α + MAIT cells were obtained from 16 HCs and 16 trauma patients. Symbols represent individual subjects and horizontal lines are median values. * $p < 0.01$ by the ANCOVA test.

Increased Plasma Levels of IL-12 and IL-18 in Trauma Patients

We measured plasma levels of IL-12 and IL-18, which are known as MAIT cell-activating cytokines [16], using ELISA in 14 trauma patients and 12 HCs. Patients with trauma showed significantly higher plasma levels of IL-12 and IL-18 as compared with HCs (median 27.2 vs. 15.1 pg/mL, $p < 0.05$; median 196.3 vs. 115.7 pg/mL, $p < 0.0005$; Fig. 3).

Activation of MAIT Cells after Stimulation with Proinflammatory Cytokines or Plasma from Trauma Patients

To determine whether MAIT cells can be activated by proinflammatory cytokines, including IL-6, IL-8, IL-12, IL-18, and TNF- α , which are elevated in the plasma of patients with trauma [21], PBMCs from trauma patients

and HCs were cultured with these recombinant cytokines consisting of IL-6, IL-8, IL-12, IL-18, and TNF- α for 24 h in the presence or absence of cytokine inhibitors (i.e., blocking antibodies against a cocktail of IL-6, IL-8, IL-12, IL-18, and TNF- α and the levels of CD69+ MAIT cells were determined by flow cytometry (Fig. 4a). Percentages of CD69+ MAIT cells were found to be significantly higher in cytokine-treated cultures than in untreated cultures (mean $\pm$ SEM 47.4 ± 2.27 vs. $1.2 \pm 0.09\%$, $p < 0.0005$, for HCs; 82.3 ± 5.88 vs. $15.2 \pm 2.49\%$, $p < 0.001$, for trauma patients, respectively) and then decreased to untreated levels after treatment with blocking antibodies (mean $\pm$ SEM 47.4 ± 2.27 vs. $1.7 \pm 0.23\%$, $p < 0.0005$, for HCs; 82.3 ± 5.88 vs. $30.4 \pm 5.15\%$, $p < 0.005$ for trauma patients, respectively; Fig. 4b).

To examine whether plasma samples obtained from trauma patients affect the activation of MAIT cells,

Fig. 2. Expression of CD69 and PD-1 and apoptosis in MAIT cells of trauma patients. Freshly isolated PBMCs were stained with FITC-conjugated anti-CD3, FITC-conjugated annexin V, APC-conjugated anti-TCR Va7.2, PE-conjugated anti-CD3, PE-conjugated anti-CD69, PE-conjugated anti-PD-1, and PE-Cy5-conjugated anti-CD161 monoclonal antibodies, and analyzed by flow cytometry. Representative percentages of CD69-expressing cells (**a**), annexin V-expressing cells (**d**), and PD-1-expressing cells (**g**)

among the MAIT cell population. Data in **b**, **e**, and **h** were obtained from 16 HCs and 16 trauma patients. Symbols represent individual subjects and horizontal lines are median values. * $p < 0.0001$ by the ANCOVA test. Relationships between MAIT cell percentages among the $\alpha\beta$ T cell population and CD69+ MAIT cells (**c**), annexin V+ MAIT cells (**f**), or PD-1+ MAIT cells (**i**) in trauma patients were determined using Spearman's correlation analysis.

PBMCs from HCs were cultured with plasma derived from the patients for 3 days in the presence or absence of the cytokine inhibitors and then CD69+ MAIT cell levels were determined by flow cytometry (Fig. 4c). Percentages of CD69+ MAIT cells were higher in the presence than in the absence of plasma (mean $\pm$ SEM 5.5 ± 0.90 vs. $2.8 \pm 0.66\%$, $p < 0.001$) and were normalized to untreated levels after treatment with blocking antibodies (mean $\pm$ SEM 5.5 ± 0.90 vs. $3.6 \pm 0.58\%$, $p < 0.005$; Fig. 4d).

Impaired $TNF-\alpha$ Production of MAIT Cells by Restimulation

Population dynamics of invariant natural killer T (iNKT) cells in response to α -galactosylceramide (α -GalCer) is characterized by prompt activation and rapid cytokine production with an initial burst of IL-4 (peaks at 2 h after stimulation) followed by IFN- γ (peaks at 24 h after stimulation) [22]. However, the production of these cytokines gradually diminished to very low levels

Fig. 3. Plasma levels of IL-12 and IL-18 in trauma patients. Plasma samples of patients were collected before specific treatment on admission. Plasma levels of IL-12 (**a**) and IL-18 (**b**) were determined by ELISA. Data were obtained from 12 HCs and 14 trauma pa-

tients. Symbols represent individual subjects and horizontal lines indicate median values. * $p < 0.05$, ** $p < 0.0005$ by the Mann-Whitney U test.

at 72 h after stimulation. These α -GalCer-experienced iNKT cells exhibit functional defects upon α -GalCer restimulation [23]. We speculated that the population dynamics of MAIT cells simulated iNKT cell behavior.

To determine the kinetics of cytokine production in MAIT cells after stimulation, PBMCs from HCs were cultured with PMA and IM and the expression of IFN- γ and TNF- α in the MAIT cell populations was examined at the single-cell level by intracellular flow cytometry (Fig. 5a). Production of TNF- α by MAIT cells in response to PMA and IM was found to peak at 0.5 h up to 1 h after stimulation and rapidly diminish to very low level at 4 h after stimulation. On the other hand, the percentages of IFN- γ + MAIT cells in response to PMA and IM were found to peak at 0.5 h up to 4 h after stimulation and gradually diminish to a low level at 36 h.

To investigate whether stimulated MAIT cells exhibited functional defects upon restimulation, PBMCs from HCs were cultured with CD3/CD28 beads and a cytokine cocktail for 16 h and washed, and then rested for 1 day. After resting, the cells were restimulated with CD3/CD28 beads and a cytokine cocktail, including IL-6, IL-8, IL-12, IL-18, and TNF- α , for 16 h, and the levels of IFN- γ + and TNF- α + MAIT cells were determined by intracellular flow cytometry (Fig. 5b). Upon restimulation, the production of TNF- α by MAIT cells was found to significantly diminish, whereas IFN- γ synthesis remained unchanged (stimulation versus restimulation for TNF- α : mean $\pm$ SEM 24.5 ± 3.64 vs. $7.2 \pm 0.55\%$, $p < 0.05$; Fig. 5c, d).

Discussion

To the best of our knowledge, this is the first study to assess the function of MAIT cells in trauma patients. The present study showed that the production of TNF- α by MAIT cells was decreased in trauma patients, together with the elevated expression of CD69. In addition, the expression of CD69 was negatively correlated with MAIT cell frequency. These patients had higher plasma levels of IL-12 and IL-18. In particular, the CD69 expression of MAIT cells was increased by stimulation with IL-18 in synergy with other proinflammatory cytokines or plasma of trauma patients and then normalized after treatment with blocking antibodies, suggesting that these cytokines may contribute to MAIT cell activation. Interestingly, the kinetics of cytokine production in MAIT cells varied between IFN- γ and TNF- α . The production of TNF- α by MAIT cells was characterized by an initial burst and rapid decline, in contrast to the delayed and sustained production of IFN- γ . Furthermore, activated MAIT cells resulted in a decline in the production of TNF- α upon restimulation. Overall, these findings suggest that an impaired TNF- α production of MAIT cells may be related to innate immune response of patients with trauma.

The present study showed that TNF- α production by MAIT cells was diminished in trauma patients, but their capacity for IFN- γ production was preserved, which is consistent with the previous data involving scrub typhus, another acute infectious disease [24]. Conversely, MAIT cells in patients with chronic HIV-1 infection and

Fig. 4. Effect of stimulation with a proinflammatory cytokine cocktail or patient plasma on the CD69 expression of MAIT cells. **a** PBMCs (1×10^6 /well) from HCs and trauma patients were incubated for 16 h in the presence or absence of cytokine inhibitors (i.e., blocking antibodies against a cocktail of IL-6, IL-8, IL-12, IL-18, and TNF- α) and then stimulated with the cytokine cocktail for 24 h. Data in **b** were obtained from 4 HCs and 4 trauma patients. **c** PBMCs (1×10^6 /well) from HCs were incubated for 16 h in the presence or absence of the cytokine inhibitors and then stimulated

for 3 days in the presence of plasma from trauma patients. Stimulated cells were stained with FITC-conjugated anti-CD3, APC-conjugated anti-TCR Va7.2, PE-conjugated anti-CD69, and PE-Cy5-conjugated anti-CD161 monoclonal antibodies. Representative percentages of CD69-expressing cells among the MAIT cell population. Data in **d** were obtained from plasma of 6 trauma patients. Values are expressed as the mean $\pm$ SEM. * $p < 0.005$, ** $p < 0.001$, *** $p < 0.0005$ by the paired t test.

Sjögren's syndrome displayed an additional defect in IFN- γ production, together with the impaired TNF- α production [25, 26]. Furthermore, a defective IFN- γ production by MAIT cells has also been observed in other chronic inflammatory or infectious diseases, such as systemic lupus erythematosus (SLE) and tuberculosis [20, 27]. These findings indicate that the differences in MAIT cell dysfunction may be linked to chronic disease, which is possibly explained by the sequential increase in multifunctional defects involving cytokine production by MAIT cells with progressive disease. Under persistent stimulation, MAIT cells may go through a sequential process of dysfunction in cytokine production (i.e., loss of TNF- α synthesis followed by IFN- γ loss), similar to T cell exhaustion [28]. Interestingly, recent studies have indi-

cated a critical role of plasma proinflammatory cytokines, such as TNF- α , IL-1, and IFN- γ in the induction of post-traumatic apoptosis, resulting in post-traumatic cardiac dysfunction and even MODS [29–31]. Taken together, these findings suggest that defective synthesis of TNF- α by MAIT cells in trauma patients may represent a physiological negative-feedback mechanism, resulting in protection against excessive inflammation.

Based on the duration and extent of activation, effector cells go through immune activation as follows: (i) early activation (e.g., CD69), (ii) chronic activation (e.g., CD38 and HLA-DR), (iii) exhaustion (e.g., PD-1, TIM-3, LAG-3, and CTLA-4), and (iv) senescence (i.e., CD57) [28, 32–36]. In the present study, MAIT cells in trauma patients exhibited a higher frequency of CD69 expression without up-

Fig. 5. Reduced TNF- α production of MAIT cells by restimulation. **a** Time course of IFN- γ and TNF- α production during MAIT cell stimulation with PMA (100 ng/mL) and IM (1 μ M). **b–d** PBMCs (1×10^6 /well) from 3 HCs were stimulated with CD3/CD28 beads and a cytokine cocktail for 16 h, washed to remove activating factors, and cultured with IL-2 (100 U/mL) to support cell survival for

1 day. Subsequently, cells were restimulated for 16 h with CD3/CD28 beads and a cytokine cocktail. Percentages of IFN- γ and TNF- α in MAIT cells were determined by intracellular flow cytometry. Values are expressed as the mean $\pm$ SEM. * p < 0.05 by the paired t test.

regulation of PD-1, in line with our previous study involving scrub typhus [24]. In contrast, CD69 and PD-1 expression of MAIT cells were upregulated in patients with chronic infectious or inflammatory diseases, such as tuberculosis, chronic HCV infection, and SLE [17, 20, 37, 38]. Furthermore, CD69 expression in MAIT cells was correlated with the ankylosing spondylitis disease activity score and SLE disease activity index in patients with ankylosing spondylitis and SLE, respectively [17, 39]. Collectively, these findings suggest that distinct profiles of activation and exhaustion markers may reflect disease activity and the degree of activation of MAIT cells in specific diseases.

Our data revealed that the plasma levels of IL-12 and IL-18 were elevated in trauma patients, which is consistent with other studies reporting that plasma levels of

proinflammatory cytokines, such as IL-1 β , IL-2, IL-6, IL-8, IL-12, IL-18, TNF- α , and IFN- γ , were significantly increased in trauma patients with seldom detectable levels of IL-2 and IFN- γ [21, 40]. Furthermore, our study demonstrated that unstimulated MAIT cells in healthy individuals were activated in response to stimulation with plasma from trauma patients or exogenous proinflammatory cytokines, including IL-6, IL-8, IL-12, IL-18, and TNF- α . The MAIT cell activation was normalized after treatment with blocking antibodies against the cytokines. However, it remains unclear which cytokines drive the CD69 upregulation. The CD69 upregulation by MAIT cells in response to cytokines was significantly blocked by each of anti-IL-12, anti-IL-18, and anti-TNF- α antibodies, and not by anti-IL-6 and anti-IL-8 antibodies, indi-

cating that IL-12, IL-18, and TNF- α are responsible for the CD69 upregulation (see online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000499343). Additional analysis including trauma patients showed that the activation behavior of patient-derived MAIT cells was similar to that of healthy individuals. The only difference is that the baseline activation level is higher in the patients than in healthy individuals. Recent reports show that MAIT cells, which are known to highly express IL-18R α and IL-12R, can be activated by IFN- α , IL-15, and IL-12+IL-18 in the absence of exogenous antigens, the concentrations of which are positively correlated with the expression of CD69 on MAIT cells [15–17]. In our study, the CD69 upregulation by patient plasma was not completely blocked by anti-cytokine antibodies against IL-6, IL-8, IL-12, IL-18, and TNF- α , suggesting that other plasma cytokines or factors besides those five cytokines may drive the response. Collectively, these findings indicate that the activation of MAIT cells in trauma is mediated via increased expression of plasma proinflammatory cytokines, such as IL-12, IL-18, and TNF- α , in an MR1-independent manner.

The mechanism of defective TNF- α production in circulating MAIT cells of patients with trauma remains to be elucidated. A possible explanation is that this dysfunction may be due to the different kinetics of release of each cytokine from the MAIT cells, which is supported by our in vitro study showing differential secretion of IFN- γ and TNF- α from MAIT cells. The different kinetics of IFN- γ and TNF- α secretions was also observed in a traumatized rat model [31]. Furthermore, MAIT cells exposed to a traumatic environment may rapidly lose the ability to produce TNF- α . This hypothesis is corroborated by our data showing that stimulated MAIT cells exhibit a functional defect in the production of TNF- α upon restimulation. Further studies are needed to investigate the molecular and signaling pathways directly mediating the impaired TNF- α production in MAIT cells of trauma patients.

Conclusions

The present study demonstrates that circulating MAIT cells are activated and functionally impaired in TNF- α production of patients with trauma. In addition, the activation and dysfunction of MAIT cells were mediated via proinflammatory cytokines. These findings provide important insight into the innate immune response of patients sustaining trauma.

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Statement of Ethics

The study protocol was approved by the Institutional Review Board of Chonnam National University Hospital, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Disclosure Statement

The authors declare that they have no competing interests.

Author Contributions

Y.-G.J., H.-M.J., Y.-N.C., J.-C.K., S.-J.K., and Y.-W.P. designed the study, collected clinical information, analyzed the raw data, performed the statistical analysis, and contributed to writing the paper. Y.-G.J., H.-M.J., and Y.-N.C. performed the experiments. All the authors read and approved the final version of the manuscript.

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