

Original Article

Dynamic changes of circulating T-helper cell subsets following severe thoracic trauma

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Abstract: Major trauma induces profound immune dysfunction, which subsequently results in sepsis and multiple organ dysfunction syndromes (MODS). The functionally conducive immune cells are of paramount important in the early recovery and development of the post-traumatic organ failure. In this study, we investigated the immune regulation after severe trauma by means of detecting the differentiation of CD4⁺ T cells. Seven male patients with thoracic trauma (aged 29.8 ± 7.6 years) hospitalized in the Intensive Care Units (ICU) in our hospital were enrolled in the study. Peripheral blood was collected from all the patients on the 1st, 7th, 14th and 21st day of admission, respectively. Flow cytometry was carried out to determine the percentage of CD4⁺ T cells differentiated into Th1, Th2, Th17 and Treg subsets, based on which the ratios of Th1/Th2 and Th17/Treg were also calculated. Twenty-five healthy male individuals (aged 34 ± 7 years) in the hospital in the same period of time served as controls. The frequencies of all the four subsets in the traumatic patients showed significant dynamic changes compared with those of the controls at the defined time points. The ratios of Th1/Th2 and Th17/Treg showed significant decrease at the study interval. Notably, the value of Th1/Th2 was significantly higher ($P=0.004$) in the trauma group than that of control group on the 1st day after admission, which was reversed on the 14th day ($P=0.014$). The imbalance of Th1/Th2 and Th17/Treg at the present study all reflected the immune dysfunction of CD4 T cells followed by the severe thoracic trauma.

Keywords: Trauma, Th1, Th2, Th17, Treg, immunological dysfunction

Introduction

Major trauma usually induces immunological system disorders, which consequently result in post-traumatic complications such as systemic inflammatory response syndrome (SIRS), sepsis and subsequent multiple organ dysfunction syndrome (MODS) [1]. The management of severe trauma is highly depends on the balance of the pro- and anti-inflammatory immune response. Aberrant alterations to the immune system are considered as key factors in the development of multiple organ failure and post-traumatic complications, which are associated with the excessive systemic inflammatory response and the critical imbalance of cell-regulated immunity.

Upon major trauma, the central regulation of the immune response is altered, which is at

least in part attributed to the impaired interaction between T lymphocytes and antigen-presenting cells [2]. Numerous reports indicated that peripheral blood monocytes could not generate T lymphocytes proliferative response in trauma patients [3]. The pattern of T-helper cells type 1 (Th1) and type 2 (Th2) has been acknowledged as an essential principle of T-cell responses [4]. To date, extensive studies have been carried out to investigate the effects of trauma on the disorder of immune system. For instance, Th1-type immune responses were markedly reduced after trauma [5]. Besides, trauma or surgery-associated physical injury increased the expression of Th2 cells and brought about impaired cell mediated immunity [6]. Further, traumatic patients showed increased susceptibility to post-traumatic complications, which consequently contributed to the imbalance of Th1/Th2 and a skewing elevation

Table 1. Baseline demographic and clinical characteristics of subjects in the study

Variables	Patients (n=7)
Age (year)	29.8 ± 7.6
Gender (male/female)	7/0
WBC (week 0) (4.0-10.0) × 10 ⁹ /L	9.53 ± 2.29
WBC (week 3) (4.0-10.0) × 10 ⁹ /L	6.10 ± 1.33
Lymphocytes (week 0) (0.8-4.0) × 10 ⁹ /L	0.59 ± 0.30
Lymphocytes (week 3) (0.8-4.0) × 10 ⁹ /L	1.15 ± 0.30
Oxygen index (mmHg) ¹	120.6 ± 22.8
Shock index ²	1.63 ± 0.26
APACH II evaluation ³	11.14 ± 1.95
AIS evaluation	
Pulmonary contusion ⁴	3.57 ± 0.53
Subcutaneous injury ⁵	1.86 ± 0.38
Hemothorax and pneumothorax ⁶	2.71 ± 0.49
Ribs fracture ⁷	2.57 ± 0.79
Complications	
ARDS (cases)	7
Hemorrhagic Shock (cases)	7
Pulmonary abscess (cases)	3
Treatment	
Vasoactive drugs + Fluid resuscitation	7
Mechanical ventilation (cases)	7
Closed thoracic drainage (cases)	7
Surgery (cases)	4
Prognosis	
Average length of stay (days)	23.9 ± 6.5
In-hospital mortality	0%

¹Lowest oxygen index within 24 hours after hospitalization. ²Highest shock index within 24 hours after hospitalization. ³Highest APACH II evaluation within 24 hours after hospitalization. ⁴Lung contusion: 441407.2 (unilateral, mild, ≤1 lobe); 441408.3 (unilateral, severe, 1-3 lobe). Lung laceration: 441431.3 (unilateral, mild, ≤1 lobe); 441432.4 (unilateral, severe, 1-3 lobe). ⁵Subcutaneous muscle injury: 410202.1 (abrasion), 410402.1 (contusion, hematoma), 410602.1 (superficial laceration), 410604.2 (length >20 cm, deep into subcutaneous tissue), 410802.1 (deletion of superficial tissue ≤100 cm²), 410804.2 (tissue deletion >100 cm²). ⁶Hemo-pneumothorax: 442200.3 (hemothorax, unilateral <1000 ml), 442202.2 (pneumothorax, unilateral pulmonary compression <50%, without durative lung leakage), 442205.3 (hemopneumothorax, unilateral <1000 ml). ⁷Rib fracture: 450201.1 (unilateral, one rib fracture), 450202.2 (unilateral, two ribs fracture), 450203.3 (unilateral, more than 3 ribs fracture).

of Th2 cells or anti-inflammatory immune reactivity [7, 8].

Accumulating evidence reveals Th17 and regulatory T cells (Tregs) also play prominent roles in modulating the host immune response following trauma [9, 10]. Recently studies indicated these T cell subsets involved in the host response to trauma. For instance, Th17 cells

were up-regulated in the spleens of rats with the traumatic optic neuropathy [11]. Choileain et al showed that it could amplify Tregs activity and contributed to the suppression of Th1 cells after severe injury [12]. In addition, Tregs played a vital role in suppressing Th1 cytokine and producing higher levels of Th2 cytokines responses after injury [8]. However, these studies are carried out in post-traumatic animal models, which varied greatly in the physiological function compared with *in vivo* conditions. Moreover, these studies are cross-sectional studies with absence of dynamic changes and accurate evaluation of cellular immunity. In this study, we aim to investigate the frequencies of Th1, Th2, Th17 and Treg subsets, as well as the dynamic changes of Th1/Th2 and Th17/Treg ratio, based on which to develop appropriate therapeutic strategies on the posttraumatic complications by inducing T cell-mediated immune responses following trauma.

Materials and methods

Patients

Seven patients with thoracic trauma admitted in the intensive care unit (ICU) of our hospital from June 2012 to September 2012 were included in this study. Patients received immunosuppressive drugs were excluded from this study. All patients received adequate oral nourishment and calorie intake in the ICU. In addition, symptomatic treatment was performed according to the specific conditions of the patients, including fixation of multiple rib fracture and flail

chest using chest strap or even internal fixation combined with mechanical ventilation; fluid resuscitation, hemostasis and blood transfusion for hemorrhagic shock; thoracic closed drainage or even continuous negative pressure suction for hemopneumothorax; oxygen inhalation or even mechanical ventilation for patients with acute respiratory distress syndrome (ARDS). The demographic information of

patients was listed in **Table 1**. Twenty-five matched healthy male individuals (34 ± 7 year-old) of the same period served as control. Written informed consents were obtained from each subject. The study was carried out with the approval of Ethics Committee of the First Affiliated Hospital of College Medical of Zhejiang University.

Preparation of human peripheral blood mononuclear cells

Peripheral blood was collected from each participant. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density-gradient centrifugation (Biochrom, Berlin, Germany), and suspended at indicated concentrations in RPMI-1640 (Thermo Electron, Waltham, MA, USA).

Flow cytometry

For Th1, Th2 and Th17 analysis, PBMCs were re-stimulated for 6 h with cell stimulation cocktail containing 50 ng/mL protein transport inhibitors, 1 mg/mL ionomycin and 1.7 mg/mL monensin (Sigma, St Louis, MO) according to the manufacturer's instructions. Subsequently, cells were extracellularly stained with PE anti-human CD8 and Pcy5 anti-human CD3 (Beckman Coulter Immunotech, Marseilles, France) at 4°C for 30 min. After surface staining, the cells were consecutively fixed and permeabilized with Fix & Perm Reagent (eBioscience, San Diego, CA) for intracellular staining with FITC antihuman INF- γ (detection of Th1 cells), FITC antihuman IL-4 (detection of Th2 cells) and FITC antihuman IL-17 antibody (detection of Th17 cells, all eBioscience, San Diego, CA, USA). The CD4⁺ cells were identified based on the expression of CD3⁺CD8⁻ Markers. For the analysis of Tregs, cell surface staining was performed with FITC-conjugated anti-CD25, PE-conjugated anti-CD127 (Beckman Coulter Immunotech, Marseille, France), and PE-cy5-conjugated anti-CD4 (eBioscience, San Diego, CA, USA). Isotype controls (eBioscience, San Diego, CA, USA) were given for compensation and confirmation of antibody specificity. The cells were incubated with the antibodies for 20 min at room temperature in the dark, followed by washing in phosphate buffered solution (PBS). The frequencies of Tregs (CD4⁺CD25⁺CD127^{low/-}), Th1 (CD3⁺CD8⁻INF- γ ⁺), Th2 (CD3⁺CD8⁻IL-4⁺), Th17 (CD3⁺CD8⁻IL-17⁺) cells

were expressed as a percentage of CD4⁺ T cells by sequential gating for lymphocytes.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Measured data were represented as mean \pm standard deviation, and descriptive statistics were represented as medians (range), or number (percentage). Mann-Whitney test was used to determine the difference between patients and healthy control. Wilcoxon test were used to compare sequential data during follow-up. $P < 0.05$ was considered statistically significant.

Results

Dynamic changes of the frequencies of Th1, Th2 cells and Th1/Th2 ratio in patients with severe trauma

Figure 1 showed the flow cytometry results of Th1 and Th2 cells. The percentage of T helper lymphocytes producing IL-4 was increased in traumatic patients, while the level of INF- γ increased initially and then decreased at the interval of trial compared with healthy individuals (**Figure 1A**). Significant increase was identified in the frequency of Th1 cells in patients with traumatic injury on day 1 after admission compared with that in the control groups ($P = 0.002$). Gradual decrease was noticed in the frequency of Th1 cells, which was significantly reduced at week 2 ($P = 0.018$, **Figure 1B**). No statistical difference was revealed in the frequency of Th2 cells in patients with traumatic injury compared with that of the healthy individuals immediately after admission. However, a significant increase was revealed in the frequency of Th2 at week 2 in patients with trauma ($P = 0.028$, **Figure 1C**). The ratio of Th1/Th2 showed significant increase in the traumatic patients compared with those in the control group on the day immediately after admission ($P = 0.004$). Nevertheless, a trend towards a decreased TH1/TH2 ratio was observed during the study. Notably, two weeks after hospitalization, the Th1/Th2 ratio showed remarkable decline in the traumatic patients compared with that of the control group ($P = 0.014$, **Figure 1D**). Taken together, suppression and disorder of T-cell immune function was induced followed by severe thoracic trauma.

Th subsets in severe trauma

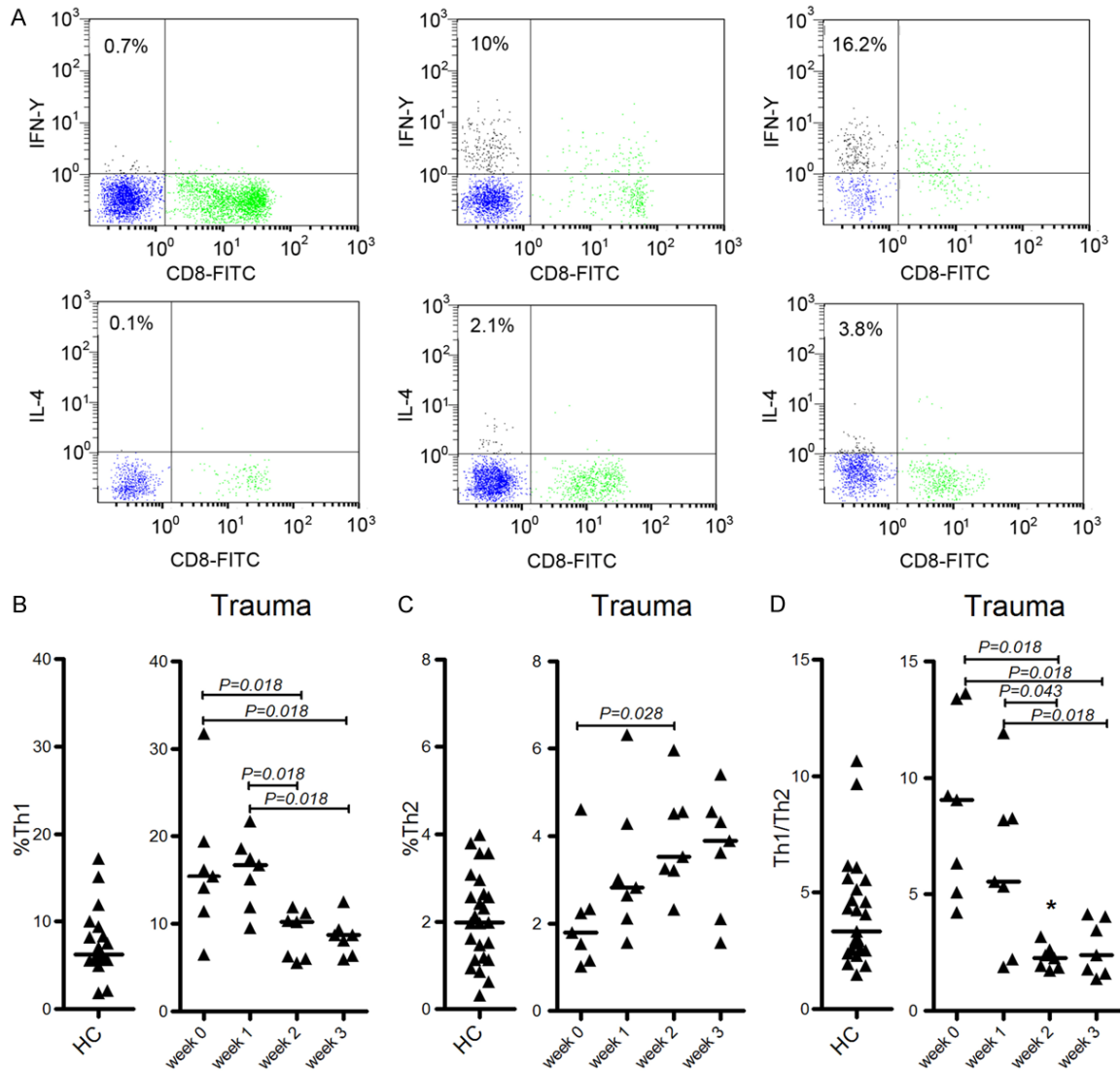


Figure 1. The dynamic change of Th1/Th2 ratio in traumatic patients and health control (HC). A: Representative dot plots of IFN- γ and IL-4 expression in peripheral CD4⁺ T cells of HC and traumatic patients. The values in the quadrants indicated the percentage of each CD4⁺ T-cell subset. B: Pooled data indicated the percentages of Th1 cells in HC and traumatic patients. C: Pooled data indicated the percentages of Th2 cells in HC and traumatic patients. D: The ratio Th1/Th2 cells presented a decreasing trend during the study and declined sharply ($P=0.014$) in the traumatic patients compared with the samples in the HC group.

Dynamic changes of the frequencies of Th17, Treg cells and Th17/Treg ratio in the traumatic patients

Figure 2 summarized the changes of the frequencies of Th17, Treg cells and Th17/Treg ratio of all the subjects. The percentage of Treg cells in the peripheral blood was higher in patients with thoracic trauma compared with that of healthy group, while the level of Th17 showed increased initially and then decreased in patients with thoracic trauma (**Figure 2A**). The

frequency of Th17 was significantly increased in traumatic patients compared to healthy controls on the day after admission ($P=0.005$). However, the frequency of Th17 cells was decreased subsequently in the trauma group (**Figure 2B**). Compared with the healthy control, the percentage of peripheral Treg cells was significantly increased in the traumatic patients on the day after admission ($P=0.015$). Meanwhile, the frequency of Treg cells was kept at a rather high level in the traumatic group within 3 weeks after admission (**Figure 2C**). In

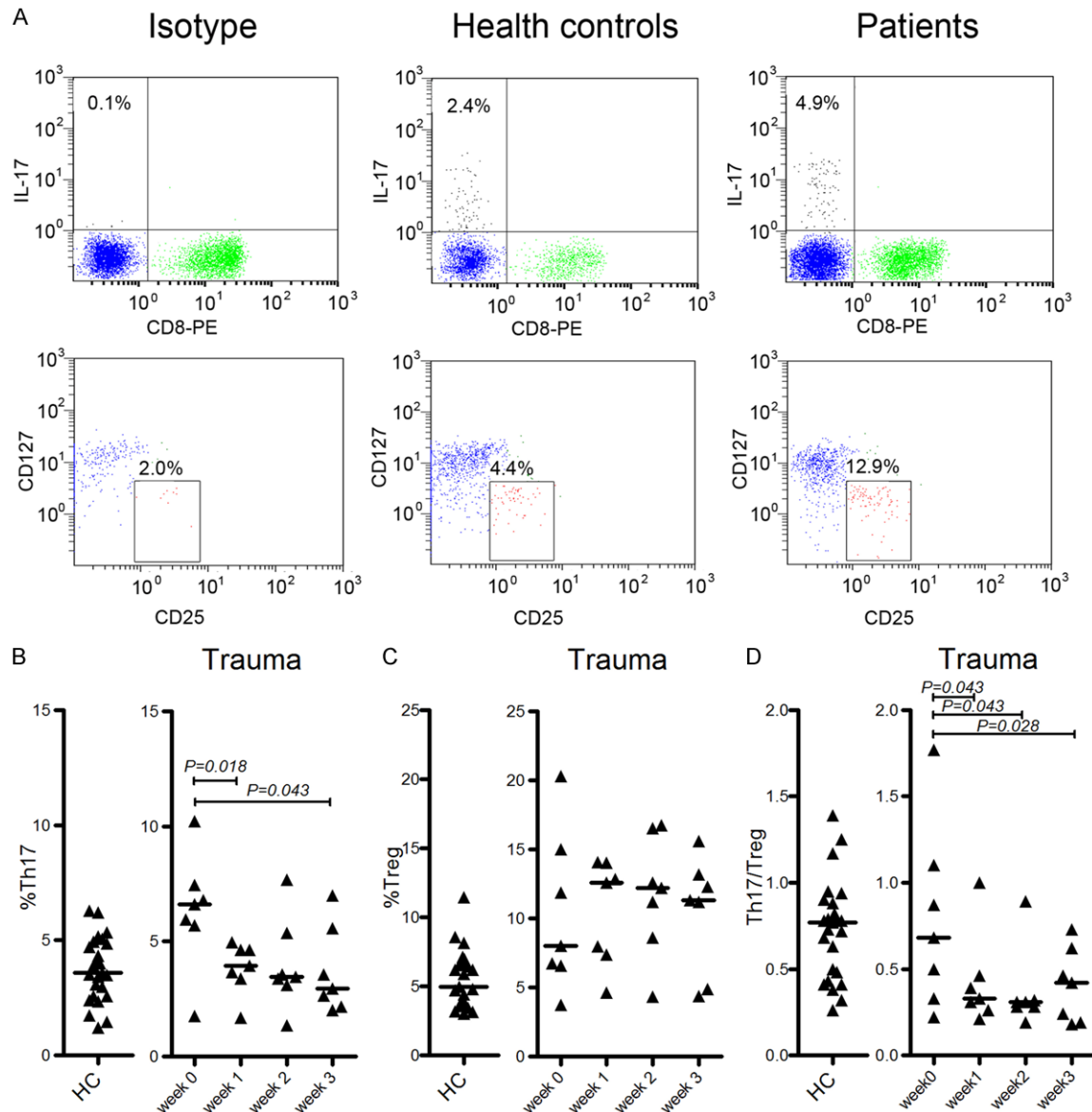


Figure 2. The dynamic change of Th17/Treg ratio in traumatic patients and HC group. A: Representative dot plots of IL-17 and CD127 expression in peripheral CD4⁺ T cells of HC subjects and traumatic patients. The values in the quadrants indicated the percentage of each CD4⁺ T-cell subset. B: Pooled data indicated the percentages of Th17 cells in HC group and traumatic patients. C: Pooled data indicated the percentages of Treg cells in HC group and traumatic patients. D: The ratio of circulating Th-17 cells to Treg cells was dramatically decreased in traumatic patients from 1st week to 3rd week after admission.

addition, no significant difference was revealed in the Th17/Treg ratio between the traumatic patients and healthy group on the day after admission as the frequencies of Th17 and Treg cells were simultaneously increased. Nevertheless, ratio of Th17/Treg was dramatically decreased in traumatic patients from the 1st week to the 3rd week after admission (**Figure 2D**). All these results indicated that thoracic injury induced the imbalance of immune system.

Discussion

Traumatic injury triggers a complex host response that disrupts immune system homeostasis required for tissue regeneration and repair. It perturbs both innate and adaptive immunity inducing the temporal change of related cells and mediators for the pro- and anti-inflammatory immune responses. These sequences of responses are thought to have significant clinical consequences since they are

responsible for the opportunistic infections and trauma-induced complications [13]. Immuno-dysregulation after major trauma is associated with high risks of sepsis and multiple organ dysfunction [14, 15]. Therefore, a complete understanding of the activation and alternation of immune response is crucial for preventing progression of complications and improving the outcome after trauma.

To date, the exact mechanism of severe trauma-associated complications is still elusive. However, there is no doubt that the suppression of cell-mediated immunity is crucial for the progression of post-traumatic complications. The immune dysfunction involving in the suppression of cell-mediated immunity may be caused by excessive activation and dysregulated recruitment of multifaceted cytokine/inhibitor profiles, macrophages with anti-inflammatory properties. In addition, the suppression or alteration of CD4⁺ T cell responses may also contribute to the development of post-traumatic immune dysfunction involving in the shift of Th1/Th2 balance toward Th2 with an accompanying loss of Th1 responses and up-regulation of Tregs [16].

In this study, we investigated the differentiation of CD4⁺ T cells into Th1, Th2, Th17 and Treg cells in patients with severe thoracic trauma. Among these CD4⁺ T cells subsets, Th1 cells promote protective cell-mediated immune responses against viruses and other intracellular pathogens with producing IL-2, IFN- γ and TNF. Th2 cells produce IL-4, IL-5 and IL-10, and drive the humoral immunity in terms of control of antibody production against extracellular organisms [17, 18]. Th17 cell is a novel subset of CD4⁺ T cells characterized by the production of IL-17 and other abundant cytokines such as IL-22, and IL-26 and TNF- α with a week and pro-inflammatory response [19]. Treg cells make up 10% of the the peripheral CD4 T cells showing major role in controlling inflammation in inflammatory disease and maintenance of immunological tolerance [20].

Accumulating evidences have indicated that T cell mediated immune function is impaired after trauma, accompanied by the suppression or alteration of CD4⁺ T cell responses [5]. Miller et al showed that Th1 response was suppressed as illustrated by the reduction of IL-2, IFN- γ , and IL-12 levels, while Th2 response was

enhanced markedly with the evaluation of IL-10 and IL-4 [2]. Meanwhile, in the major burn traumatic patients, the production of IL-4 was excessively upregulated while the IFN- γ was increased [21]. Nowadays, the Th1/Th2 balance hypothesis has been highlighted to clarify the pathophysiology underlying the post-traumatic immune response [22]. Decker et al showed that the Th1/Th2 ratio was decreased with the suppression of cell-mediated immunity after surgery [23]. In agreement with the above observations, our results indicated significant decrease of Th1 cells and increase of Th2 cells in the thoracic traumatic patients at week 2 compared with that of the baseline level. In addition, we found that trauma decreased Th1/Th2 ratio, which was consistent with the previous report compared with the samples in the control group [23]. All these suggested that the trauma potentially induced a shift in the Th1/Th2 balance toward Th2 based on the enhancement of humoral immunity and the suppression of T cell-mediate immunity, which defined a state of immune dysfunction and increased the susceptibility to sepsis and MODS complications.

Tregs and Th17 cells also seem to modulate the immune response after trauma, and the balance between them is essential for maintaining immune homeostasis. Neely indicated that the number and percentage of Th17 cells were significantly higher in burn mice than those in sham group on d3, d7, and d14 day after injury [24]. Stoecklein et al demonstrated that Tregs were activated in response to injury which driven trauma-induced suppression of Th1 responses and T cell anergy [13]. In our study, significant enhancement of frequency of Th17 cells was only identified in the peripheral blood of patients with thoracic trauma on the day after admission, while the number of Tregs was significantly elevated and maintained at a higher level at the interval of study, which contributed to the suppression of Th1 immune function accompanied by a reduced frequency of Th1 cells two weeks after trauma. In addition, we observed that Th17/Treg ratio decreased in CD4⁺ T cells in traumatic patients from the 1st week to the 3rd week after admission. Taken together, these results provided additional evidence that the immune dysfunction with the imbalance of Th17/Treg contributed to the pathogenesis and development of trauma. Meanwhile, we pointed that the sequence of

changes of the overwhelming anti-inflammatory response 1 week after trauma may impose patients on high risk of secondary traumatic complications combined the fact that three patients were induced sepsis in this study.

It is essential to adopt the effective immunotherapy since serious adverse consequences caused by immune dysregulation after trauma. To date, it has attracted a growing attention to modulate the balance of CD4⁺ T cell subsets and the expression of involved cytokines for the treatment of immune disorders. Göebel et al proposed that IL-12 treatment with low-dose could increase or maintain the production of pro- and anti-inflammatory mediators at the same time in host defence [25]. Liao et al showed that IL-2 had effect on the differentiation of T-helper cells into Th1, Th2 and Th17 cells by regulating the expression of cytokine receptors to help keep and specify the differentiated states [26]. Tian et al indicated that vitamin D played an important role in modulating the balance of Th17/Treg [27]. Moreover, histamine 4 receptor (H4R) agonist could influence the production and function of T cells and mediate Th1/Th2 balance by decreasing the expression of IL-4 and increasing the levels of IFN- γ , TNF- α and IL-1 β [28]. In the current study, it presented dynamic changes of immune function in severe injury patients with inflammatory and anti-inflammatory responses at the early and late stage after thoracic trauma, which was conducive to the selection of appropriate immune therapy at the terms of specific immune state.

In conclusion, we first described the differentiation of CD4⁺ T cells into Th1, Th2, Th17 and Treg cells in traumatic patients with the dynamic variations. The decreased Th1/Th2 and Th17/Treg ratios, increased Tregs and Th2 percentages appeared to have profound effects on the development of suppressed adaptive immunity after severe thoracic trauma. All these findings suggest an early opportunity for immune modulation and aid in the introduction of appropriate therapeutic strategies of posttraumatic complications.

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Disclosure of conflict of interest

None.

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References

- [1] Angele MK and Faist E. Immunodepression in the surgical patient and increased susceptibility to infection. *The Sepsis Text*; Springer; 2002. pp. 743-756.
- [2] Miller AC, Rashid RM and Elamin EM. The "T" in trauma: the helper T-cell response and the role of immunomodulation in trauma and burn patients. *J Trauma* 2007; 63: 1407-1417.
- [3] Heizmann O, Koeller M, Muhr G, Oertli D and Schinkel C. Th1- and Th2-type cytokines in plasma after major trauma. *J Trauma* 2008; 65: 1374-1378.
- [4] Allen JE and Maizels RM. Th1-Th2: reliable paradigm or dangerous dogma? *Immunol Today* 1997; 18: 387-392.
- [5] Murphy T, Paterson H, Rogers S, Mannick JA and Lederer JA. Use of intracellular cytokine staining and bacterial superantigen to document suppression of the adaptive immune system in injured patients. *Ann Surg* 2003; 238: 401.
- [6] Marik PE and Flemmer M. The immune response to surgery and trauma: Implications for treatment. *J Trauma Acute Care Surg* 2012; 73: 801-808.
- [7] Hsieh CH, Hsu JT, Hsieh YC, Frink M, Raju R, Hubbard WJ, Bland KI and Chaudry IH. Suppression of Activation and Costimulatory Signaling in Splenic CD4⁺ T Cells after Trauma-Hemorrhage Reduces T-Cell Function: A Mechanism of Post-Traumatic Immune Suppression. *Am J Pathol* 2009; 175: 1504-1514.
- [8] MacConmara MP, Maung AA, Fujimi S, McKenna AM, Delisle A, Lapchak PH, Rogers S, Lederer JA and Mannick JA. Increased CD4⁺CD25⁺ T regulatory cell activity in trauma patients depresses protective Th1 immunity. *Ann Surg* 2006; 244: 514.
- [9] Chen Z and O'Shea JJ. Th17 cells: a new fate for differentiating helper T cells. *Immunol Res* 2008; 41: 87-102.

- [10] Afzali B, Lombardi G, Lechler R and Lord G. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. *Clin Exp Immunol* 2007; 148: 32-46.
- [11] Zheng H, Zhang Z, Luo N, Liu Y, Chen Q and Yan H. Increased Th17 cells and IL-17 in rats with traumatic optic neuropathy. *Mol Med Reps* 2014; 10: 1954-1958.
- [12] Choileain NN, MacConmara M, Zang Y, Murphy TJ, Mannick JA and Lederer JA. Enhanced regulatory T cell activity is an element of the host response to injury. *J Immunol* 2006; 176: 225-236.
- [13] Stoecklein VM, Osuka A and Lederer JA. Trauma equals danger-damage control by the immune system. *J Leukoc Biol* 2012; 92: 539-551.
- [14] Jastrow KM III, Gonzalez EA, McGuire MF, Suliburk JW, Kozar RA, Iyengar S, Motschall DA, McKinley BA, Moore FA and Mercer DW. Early cytokine production risk stratifies trauma patients for multiple organ failure. *J Am Coll Surg* 2009; 209: 320-331.
- [15] Moore FA, Sauaia A, Moore EE, Haenel JB, Burch JM and Lezotte DC. Postinjury multiple organ failure: a bimodal phenomenon. *J Trauma* 1996; 40: 501-512.
- [16] Kimura F, Shimizu H, Yoshidome H, Ohtsuka M and Miyazaki M. Immunosuppression following surgical and traumatic injury. *Surg Today* 2010; 40: 793-808.
- [17] Webster NR and Galley HF. Immunomodulation in the critically ill. *Br J Anaesth* 2009; 103: 70-81.
- [18] Kim Y, Kang SH, Hong TH, Cho ML, Han HJ, Kwon SJ and Lee J. Effects of dexmedetomidine on the ratio of T Helper 1 to T Helper 2 cytokines in patients undergoing laparoscopic cholecystectomy. *J Clin Anesth* 2014; 26: 281-285.
- [19] Angele MK, Knöferl MW, Ayala A, Bland KI and Chaudry IH. Testosterone and estrogen differently effect Th1 and Th2 cytokine release following trauma-haemorrhage. *Cytokine* 2001; 16: 22-30.
- [20] Föhse L, Suffner J, Suhre K, Wahl B, Lindner C, Lee CW, Schmitz S, Haas JD, Lamprecht S and Koenecke C. High TCR diversity ensures optimal function and homeostasis of Foxp3+ regulatory T cells. *Eur J Immunol* 2011; 41: 3101-3113.
- [21] Zedler S, Bone RC, Baue AE, Donnersmarck GHV and Faist E. T-cell reactivity and its predictive role in immunosuppression after burns. *Crit Care Med* 1999; 27: 66-72.
- [22] Heidecke CD, Hensler T, Weighardt H, Zantl N, Wagner H, Siewert JR and Holzmann B. Selective defects of T lymphocyte function in patients with lethal intraabdominal infection. *Am J Surg* 1999; 178: 288-292.
- [23] Decker D, Schöndorf M, Bidlingmaier F, Hirner A and von Ruecker AA. Surgical stress induces a shift in the type-1/type-2 T-helper cell balance, suggesting down-regulation of cell-mediated and up-regulation of antibody-mediated immunity commensurate to the trauma. *Surgery* 1996; 119: 316-325.
- [24] Neely CJ, Maile R, Wang MJ, Vadlamudi S, Meyer AA and Cairns BA. Th17 (IFN γ -IL17+) CD4+ T cells generated after burn injury may be a novel cellular mechanism for postburn immunosuppression. *J Trauma Acute Care Surg* 2011; 70: 681-690.
- [25] Göebel A, Kavanagh E, Lyons A, Saporoschetz IB, Soberg C, Lederer JA, Mannick JA and Rodrick ML. Injury induces deficient interleukin-12 production, but interleukin-12 therapy after injury restores resistance to infection. *Ann Surg* 2000; 231: 253.
- [26] Liao W, Lin JX, Wang L, Li P and Leonard WJ. Modulation of cytokine receptors by IL-2 broadly regulates differentiation into helper T cell lineages. *Nat Immunol* 2011; 12: 551-559.
- [27] Tian A, Ma H, Cao X, Zhang R, Wang X and Wu B. Vitamin D Improves Cognitive Function and Modulates Th17/Treg Cell Balance After Hepatectomy in Mice. *Inflammation* 2015; 38: 500-509.
- [28] Ahmad SF, Zoheir KM, Ansari MA, Korashy HM, Bakheet SA, Ashour AE and Attia SM. Stimulation of the histamine 4 receptor with 4-methylhistamine modulates the effects of chronic stress on the Th1/Th2 cytokine balance. *Immunobiology* 2015; 220: 341-349.