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Original Article

The Tim-3/galectin-9 axis interferes with Th1/Th2 cell balance and induces lymphocyte apoptosis in patients with sepsis

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Abstract: Sepsis is characterized by T cell immune imbalance, particularly between Th1 and Th2 cells, and is mediated by pro- and anti-inflammatory cytokines. However, the mechanism underlying the immune regulation of sepsis remains poorly understood. Here, the mechanism of Th1/Th2 balance in patients with sepsis was investigated. Peripheral blood was collected from patients with sepsis and healthy controls and the proportion of $CD3^+CD4^+$ T cells was analyzed by flow cytometry. Soluble (s) Tim-3, galectin-9 (Gal-9), IFN-γ and IL-4 were determined by ELISA, and the mRNA expression of Tim-3, Gal-9, T-bet and GATA-3 were analyzed via RT-qPCR. Following Tim-3 silencing in lymphocytes using anti-Tim-3 antibodies, the content of IFN-γ and IL-4 was detected by ELISA. In addition, the apoptosis rate of lymphocytes was evaluated by PI/Annexin V staining, and the associations between Tim-3, Gal-9, IFN-γ and IL-4 were analyzed by Pearson's correlation coefficient. Data indicated that $Tim\text{-}3^+$ T cells were increased. Additionally, the levels of soluble Tim-3, Gal-9, IL-4 and GATA-3 were increased, while those of IFN-γ and T-bet were decreased. When the expression of Tim-3 was inhibited, the levels of IFN-γ were upregulated and the apoptosis rate of lymphocytes was reduced. However, no significant changes were observed in the expression of IL-4. Furthermore, a positive association between Tim-3 and Ga-9, and a negative association between Tim-3 and IFN-γ was identified. The current study suggested that Tim-3 combined with Gal-9 could regulate the balance of Th1/Th2 cells and induce lymphocyte apoptosis in patients with sepsis.

Keywords: Tim-3/Galectin-9, Th1/Th2 cells, IFN-γ/IL-4, sepsis, apoptosis

Introduction

Sepsis is a syndrome characterized by life-threatening multiple organ dysfunction caused by the imbalance of the body's response to pathogen infection. Sepsis is often accompanied by immune dysfunction and systemic inflammatory response syndrome, and is also characterized by a rapid onset and a high mortality [1, 2]. In addition, sepsis is considered an extremely dangerous disease. It has been reported that sepsis is commonly caused by several bacterial and viral infections, which may result in immune system disorders, eventually leading to the expression of multiple inflammatory factors and cell metabolites [3-5]. T cells play a crucial role in sepsis. Gupta et al [7] revealed the existence of a cell-mediated immune response imbalance and Th1/Th2 ratio disorder in patients with sepsis. However, although the Th1/Th2 cell imbalance has been previously reported in patients with sepsis, the molecules mediating this effect remain largely unknown.

T cell immunoglobulin domain and mucin domain (Tim) is a transmembrane protein predominantly expressed on T cells [8]. Emerging evidence has suggested that Tim triggers a variety of immune responses, including autoimmune diseases [9, 10], allergic diseases [11] and cancer [12, 13]. Tim-3, an inhibitory receptor expressed in differentiated and mature Th1 cells, is induced via the activation of members of the Tim family [8, 14]. Two forms of Tim-3 have been identified, the membrane (mTim-3) and soluble Tim-3 (sTim-3). It has been reported that galectin-9 (Gal-9) can specifically bind

Table 1. The primer sequence

Gene	Primer (5'-3')
Tim-3	Forward primer: GCAGGGCAGATAGGCATTCT
	Reverse primer: CTGCTGCTACTACTTACAAGGTC
Galectin-9	Forward primer: GTCTCCAGGACGGACTTCA
	Reverse primer: CACGTACCCTCCATCTTCA
T-bet	Forward primer: TGAGGTGAACGACGGAG
	Reverse primer: CATTCTGGTAGGCAGTCACG
GATA-3	Forward primer: AGGGAGTGTGTGAACTGTGGG
	Reverse primer: CTTCGCTTGGGCTTAATGAGG
GAPDH	Forward primer: ACAGTCAGCCGCATCTTCTT
	Reverse primer: TGGAAGATGGTGATGGGATG

with Tim-3 to induce cell activation and apoptosis [15]. More and more evidence shows that Tim-3 has an important effect on maintaining Th1/Th2 balance [16, 17].

The current study aimed to investigate whether the Th1/Th2 imbalance in patients with sepsis was associated with the Tim-3/Gal-9 axis. Therefore, the activation of the Tim-3/Gal-9 pathway and the expression of Th1- and Th2-related cytokines were evaluated *in vivo* and *in vitro* using flow cytometry, ELISA and RT-qPCR, to uncover the possible mechanism underlying Th1/Th2 imbalance in sepsis and other infectious diseases.

Materials and methods

Patients and samples

A total of 96 patients with sepsis or severe sepsis were recruited between January 2018 and January 2020 (**Table 1**). The inclusion criteria were as follows: Sequential Organ Failure Assessment (SOFA) score >2 points from baseline. SOFA score was based on several parameters, including partial pressure of oxygen/fraction of inspired oxygen concentration, platelet count, serum creatinine levels, bilirubin, urine volume and nervous system scores. In addition, all patients met the diagnostic criteria for sepsis and severe sepsis according to the Surviving Sepsis Campaign guidelines. The exclusion criteria were as follows: Patients with malignant tumors; patients with severe metabolic diseases such as diabetes; patients with autoimmune diseases and immunosuppression; patients with other allergic disorders; and patients with organ transplantation. Additionally, samples from 96 healthy individuals were obtained from the General Hospital of the Northern Theater Command and served as the control group. All the studies were approved by the ethical committees of General Hospital of the Northern Theater Command on October 28, 2018 (protocol number: 2018-74). All participants provided written informed consent. Peripheral blood and serum were collected from all participants for subsequent experiments.

Flow cytometry

A 1 × 10^6/ml cell suspension in a total volume of 100 μl PBS in an anticoagulant tube was incubated with CD3-PerCP, CD4-fluorescein isothiocyanate (FITC) and Tim-3-allophycocyanin antibodies (all from BD Pharmingen) in the dark for 30 min. Subsequently, cells were lysed with 1 × Lysing Solution (BD Biosciences) in the dark for 10 min. The suspension was centrifuged at 350 × g, the supernatant was discarded, the cells were rinsed with 1 × PBS followed by centrifugation. Finally, the supernatant was discarded, cells were resuspended in PBS, and the percentage of $CD3^+CD4^+$ Tim-3^+ cells was measured using the FACS Canto II flow cytometer (BD Biosciences).

RT-qPCR assays

The mRNA expression of Tim-3, Gal-9, T-bet, and GATA-3 were evaluated using the SYBR Green Master Mix (Thermo Fisher Scientific, Inc.) in a 25 μl reaction, in triplicate. Ct values were determined on the Bio-Rad CFX96 Sequence Detection System (Bio-Rad). The primer sequences used are listed in **Table 1**.

ELISA

The optical density (OD) values of sTim-3, Gal-9, IL-4, IFN-γ, IL-6 and TNF-α in serum and cell culture supernatants were detected using corresponding ELISA kits (R&D Systems, Inc.), according to the manufacturer's instructions. The corresponding cytokine concentration was calculated according to the standard curve.

Cell culture

Peripheral blood lymphocytes were isolated from healthy individuals and resuspended in RPMI-1640 medium containing 10% newborn

Table 2. Clinical characteristics of patients with sepsis

	Control (n=96)	Sepsis (n=96)	*P* value
Age	48 (21-81)	75 (30-89)	0.242
Sex (M/F)	53/43	50/46	-
WBC (10^9/L)	7.23±2.59	15.91±11.32	0.014
RBC (10^9/L)	4.84±0.35	3.69±0.69	0.001
HGB (g/L)	156.73±19.38	115.45±24.37	0.003
NEU (10^9/L)	4.65±2.02	15.22±11.72	<0.001
PLT (10^9/L)	209.58±66.42	121.72±79.85	<0.001
RDW (%)	13.41±5.65	15.88±7.29	>0.05
CRP (mg/L)	6.30±3.27	144.52±85.26	<0.001
PCT (ng/mL)	0.03±0.01	66.24±37.33	<0.001
IL-6 (pg/mL)	4.36±1.38	99.88±35.43	<0.001

calf serum. Subsequently, 1 × 10^6/ml lymphocytes were seeded into 6-well plates. In the present study, two groups of cells were included. Cells in the first group were supplemented with 0.1 μg/ml lipopolysaccharide (LPS group; Sigma-AldrichA), while those in the control group were treated with equal volume of PBS (PBS group). Subsequently, the cells in both groups were treated with 10 μg/ml concanavalin A (ConA; Sigma-Aldrich) and incubated at 37°C for 24 h. Cells were first treated with 10 μg/ml anti-Tim-3 monoclonal antibody (BioLegend, Inc.) for 12 h and then with 1 μg/ml Gal-9 (Abcam) for an additional 12 h. The cells and supernatants were collected for subsequent experiments.

MTT assay

Briefly, 1 × 10^5 cells/ml were seeded into 96 well plates, and incubated with 10, 100, 1,000 and 10,000 ng/ml LPS at 37°C for 24 h. Subsequently, 5 mg/ml of MTT solution (Sigma-Aldrich) was added into each well and cells were incubated for an additional 4 h. The plates were then depleted, 100 μl of DMSO was added into each well for 10 min, and the OD value in each well was measured.

PI/Annexin V staining

Briefly, 1 × 10^5 cells/ml were resuspended in cold PBS and were then stained with annexin V-FITC and PI for 15 min in the dark. Following incubation for 1 h, the cells were resuspended in PBS and analyzed using the FACS Canto II flow cytometer (BD Biosciences).

Statistical analysis

All data presented in the study were expression as means ± SD. Statistical analysis was performed by GraphPad Prism 7.0 (GraphPad Software, Inc.). Student's t test and one-way ANOVA were used to assess the different among the groups. Pearson coefficient correlation test was used to test the correlations between serial variables. A value of $P<0.05$ was considered statistically significant.

Results

Clinical characteristics of patients with sepsis

In the present study, a total of 96 patients with sepsis were enrolled, including 50 males and 46 females. The clinical characteristics of patients are presented in **Table 2**. In addition, the number of peripheral blood cells and the levels of inflammatory markers were also measured. Therefore, the count of peripheral blood white blood cells and neutrophils, and the levels of C-reactive protein, IL-6, and procalcitonin (PCT) were significantly increased. By contrast, the count of red blood cells and platelets (PLT), and the levels of hemoglobin were markedly decreased.

Levels of Tim-3 and Gal-9 in sepsis

The percentage of $CD3^+CD4^+$ Tim-3^+ T cells was determined in patients with sepsis by flow cytometry (**Figure 1A**, **1B**). Compared with the control group, the percentage of Tim-3^+ cells in patients with sepsis was increased ($P<0.001$; **Figure 1B**). The secretion levels of sTim-3 and Gal-9 were measured by ELISA and the results indicated that the levels of both sTim-3 and Gal-9 were significantly increased in sepsis ($P<0.001$, **Figure 2A**, **2B**). Furthermore, the mRNA expression of Tim-3 and Gal-9 were detected by RT-qPCR. Therefore, both Tim-3 and Gal-9 were also upregulated in patients with sepsis ($P<0.001$; **Figure 2C**, **2D**).

Th1/Th2 imbalance in sepsis

ELISA was employed to measure the secretion levels of the Th1/Th2-related cytokines. The results revealed that the levels of the Th1 cytokine, IFN-γ, were significantly diminished ($P<0.001$; **Figure 3A**), while those of the Th2-related cytokine, IL-4, were notably increased

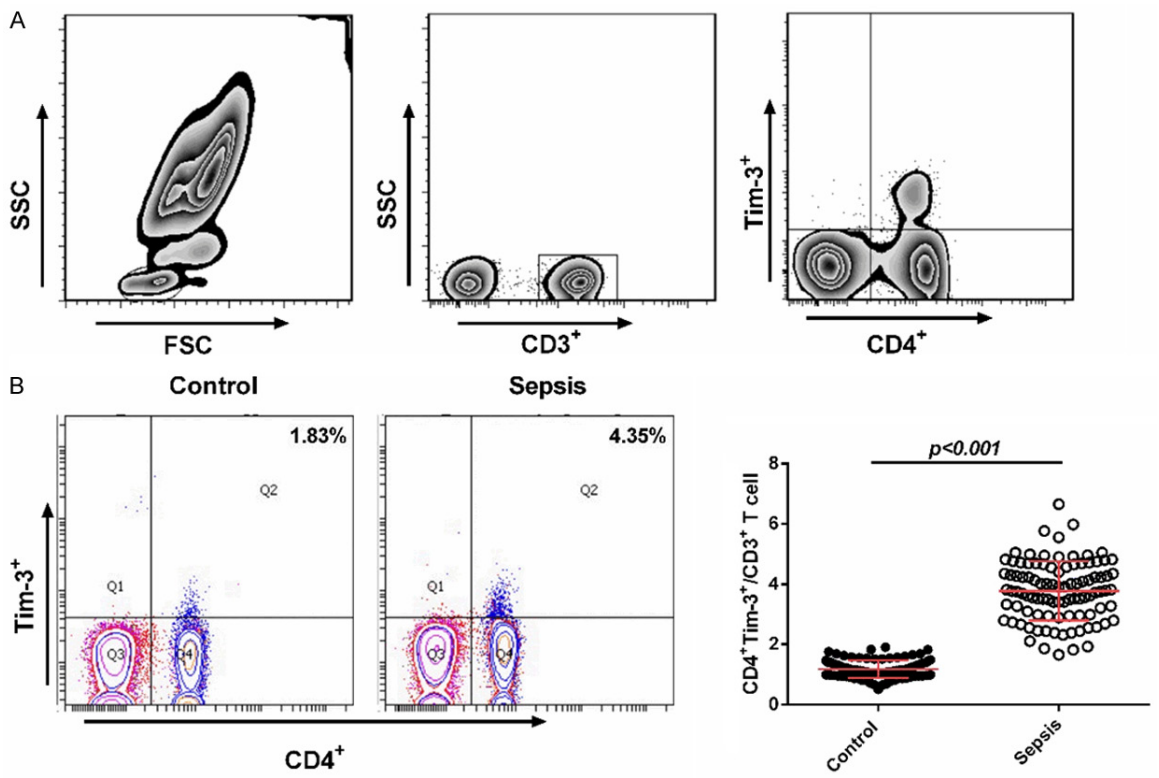

Figure 1. Gating strategy for flow cytometric analysis and proportion of $CD3^+CD4^+$ Tim-3^+ cells. A: FSC intensity is associated with cell size, and SSC with cell granularity. Following gating for CD3 lymphocytes, the gating for Tim-3^+ cells in patients with sepsis and healthy controls are depicted. B: T cell Tim-3^+ cell population is shown.

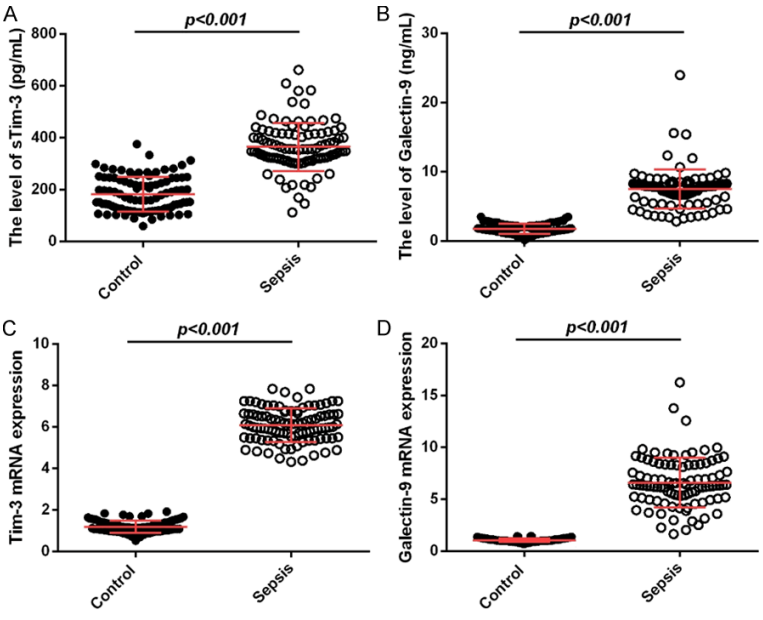

Figure 2. mRNA and secretion levels of Tim-3 and Gal-9. The secretion levels of (A) sTim-3 and (B) Gal-9 are shown. The mRNA expression levels of (C) Tim-3 and (D) Gal-9 are shown. sTim-3, soluble Tim-3.

in sepsis (P<0.001; **Figure 3B**). Additionally, the IFN-γ/IL-4 ratio was also decreased (P<0.001; **Figure 3C**). The results of RT-qPCR demonstrated that T-bet was downregulated (P<0.001; **Figure 3D**), while GATA-3 was upregulated in patients with sepsis (P<0.001; **Figure 3E**). The T-bet/GATA-3 ratio was also notably reduced (P< 0.001; **Figure 3F**).

Tim-3/Gal-9 regulates the balance of Th1/Th2 cells in sepsis

Following stimulation with LPS, the cell culture supernatants and cells were collected, and cell survival was evaluated by MTT assay. The content of the inflammatory cytokines, IL-6 and TNF-α, was measured in cell culture supernatants by ELISA. A concentration of 0.1 μg/ml LPS was chosen to stimulate cells (**Figure 4A-C**). A sepsis simulation *in vitro* model was established,

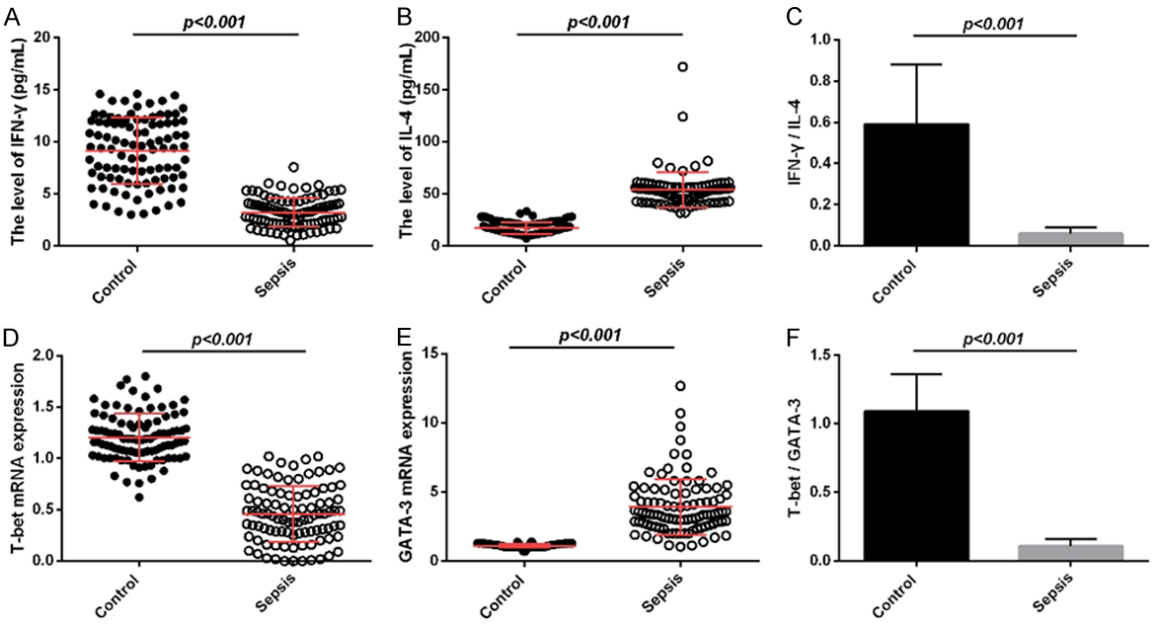

Figure 3. IFN-γ (Th1) and IL-4 (Th2) cytokine expression levels, and the mRNA expression levels of the transcription factors T-bet (Th1) and GATA-3 (Th2). The secretion levels of (A) IFN-γ and (B) IL-4 are shown. (C) The IFN-γ/IL-4 ratio is shown. The mRNA expression levels of (D) T-bet and (E) GATA-3 are shown. (F) The T-bet/GATA-3 ratio is shown.

and the culture supernatants of cells treated with PBS, LPS, LPS + Gal-9, LPS + Tim-3 + Gal-9, anti-Tim-3 antibody or Gal-9 were collected to measure the levels of the Th1/Th2-related cytokines using ELISA. The secretory levels of IFN-γ were significantly increased after LPS stimulation (**Figure 4D**), while they were notably reduced following stimulation with LPS + Gal-9. Cell stimulation with Gal-9 had no effect on the secretion levels of IFN-γ. Interestingly, no statistically significant changes were observed in IL-4 levels among different treatment groups (**Figure 4E**). Furthermore, to investigate whether the Tim-3/Gal-9 axis could mediate the Th1/Th2 imbalance in septic patients, Tim-3 expression was inhibited using an anti-Tim-3 monoclonal antibody. Therefore, following treatment of cells with anti-Tim-3 antibody, the secretion of IFN-γ was enhanced in cells stimulated with Gal-9. However, no differences were observed in the secretion levels of IL-4.

Tim-3/Gal-9 induces lymphocyte apoptosis

The apoptosis rate of lymphocytes was assessed. Data indicated that the apoptosis of lymphocytes was enhanced after cell stimulation with LPS (**Figure 5**). Additionally, lymphocyte apoptosis was also increased following cell stimulation with LPS + Gal-9. By contrast, treatment of cells with anti-Tim-3 antibody significantly attenuated apoptosis. However, stimulation with Gal-9 or Tim-3 alone, slightly increased cell apoptosis.

Association of Tim-3/Gal-9 with IFN-γ and IL-4

Subsequently, the association between Tim-3/Gal-9 and the expression of IFN-γ and IL-4 was evaluated in patients with sepsis. $P<0.05$ and $r>0.5$ were considered to indicate a statistically significant association. The results revealed a significant positive association between Tim-3 and Gal-9 ($r=0.697$; $P<0.0001$; **Figure 6A**). In addition, Tim-3 was negatively associated with IFN-γ ($r=-0.543$; $P<0.0001$; **Figure 6B**). However, there was no significant association between Tim-3 and IL-4 ($r=0.326$; $P=0.0012$; **Figure 6C**), and between Gal-9 and IFN-γ ($r=-0.445$; $P<0.001$; **Figure 6D**) and IL-4 ($r=0.130$; $P=0.208$; **Figure 6E**).

Discussion

Sepsis is mainly characterized by the imbalance between the inflammatory and anti-inflammatory responses. Once the balance is destroyed, the disease often aggravates [1]. As an important component of the adaptive

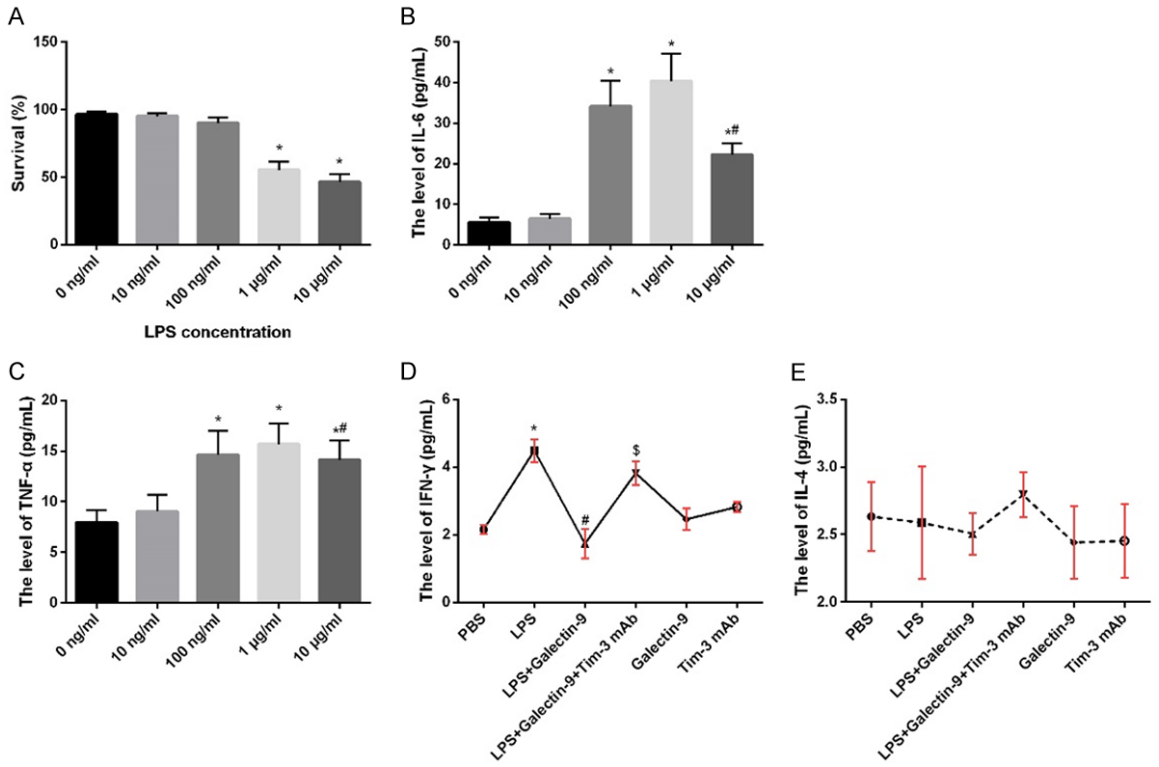

Figure 4. Tim-3/Gal-9 axis regulates the Th1/Th2 balance in sepsis. A: Lymphocytes were isolated and treated with different concentrations of LPS to induce lymphocyte inflammation *in vitro*. MTT assay was carried out to detect the effect of different concentrations of LPS on the survival of lymphocytes. B: Following stimulation of lymphocytes with different concentrations of LPS, the levels of IL-6 in cell culture supernatants were measured using ELISA. C: Following stimulation of lymphocytes with different concentrations of LPS, the levels of TNF-α in cell culture supernatants were detected by ELISA. D: Following treatment with LPS and ConA, the isolated lymphocytes were cultured in the presence of Gal-9 and anti-Tim-3 antibody, respectively, and the secretion levels of IFN-γ in the cell culture supernatants were determined. E: Following treatment with LPS and ConA, the isolated lymphocytes were cultured in the presence of Gal-9 and anti-Tim-3 antibody, respectively, and the secretion levels of IL-4 were detected in the cell culture supernatants. $^{*}P<0.05$, compared with the PBS group; $^{\#}P<0.05$, compared with the LPS group; $^{\$}P<0.05$, compared with the LPS + Gal-9 group.

immune responses, T cells, are involved in the pathophysiology of several diseases. Herein, peripheral blood cells and serum were collected from patients with sepsis. Subsequently, the secretion and mRNA levels of Th1/Th2-related cytokines were determined in serum and peripheral blood cells, respectively. A Th1/Th2 cell imbalance was revealed in patients with sepsis. However, the mechanism underlying this imbalance remains unclear.

The activation of T cells depends on costimulatory molecules, such as CD28 and CTLA-4 [18]. After receiving the stimulatory signal, T cells can play an immune-stimulatory or immunosuppressive role [18]. Tim-3 is a negative costimulatory molecule, particularly expressed on Th1 cells, involved in the Th1-mediated immune responses [19, 20]. Additionally, Gal-9, expressed by different types of cells, belongs to soluble glycoproteins and adheres to the cell membrane to induce cell apoptosis in thymocytes, and external $CD4^{+}$ and $CD8^{+}$ cells [21]. Galactose lectin-9 is the only known Tim-3 ligand. Tim-3 binds to its ligand and transmits negative stimulatory signals to promote immune tolerance in response to excessive immune response, thus ensuring a balanced immune response [22, 23]. Li *et al* [24] demonstrated that $Tim3^{+}$ T cells could interact with Gal-9-expressing T regulatory cells to inhibit the Th1 cell-mediated response in patients with osteosarcoma. Herein, the proportion of $Tim\text{-}3^{+}$ T cells was measured in patients with sepsis.

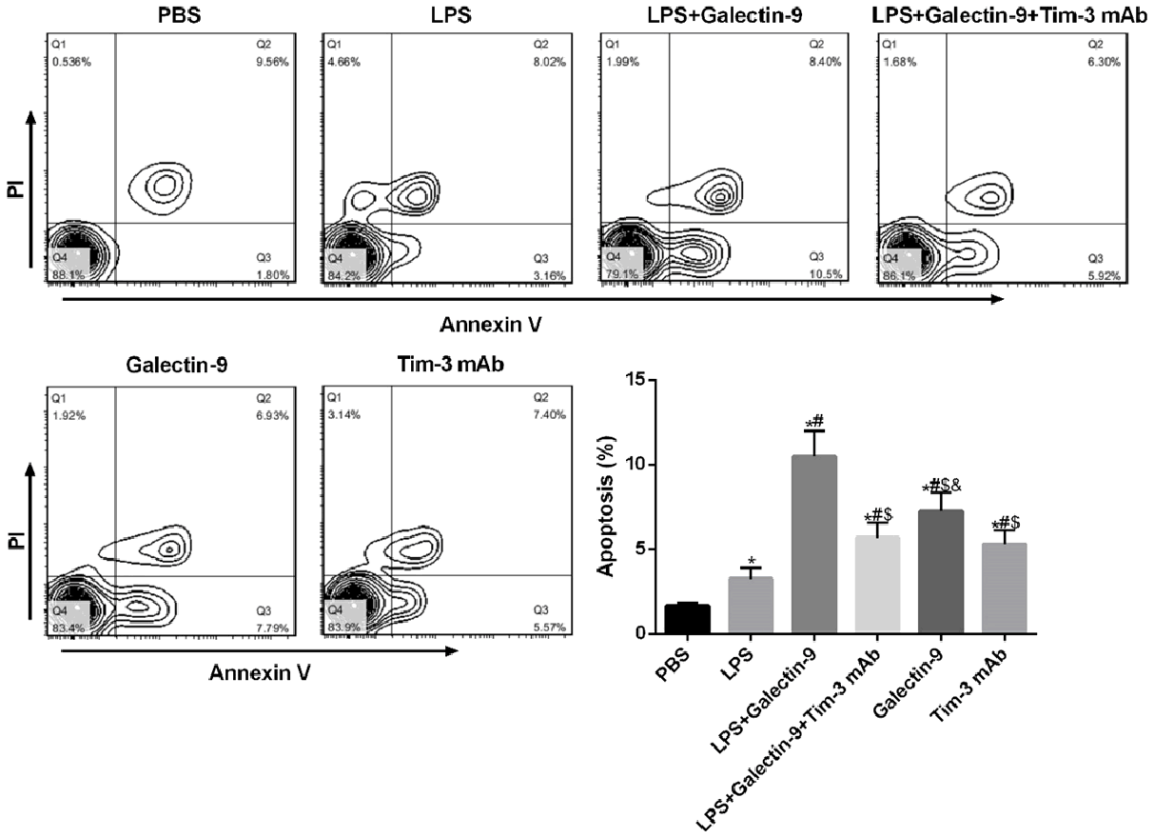

Figure 5. Tim-3/Gal-9 axis induces lymphocyte apoptosis. The apoptosis rate of lymphocytes stimulated with LPS, LPS + Gal-9, or LPS + Gal-9 + anti-Tim-3 antibody was assessed. $^{*}P<0.05$, compared with the PBS group; $^{\#}P<0.05$, compared with the LPS group; $^{\$}P<0.05$, compared with the LPS + Gal-9 group; $^{\&}P<0.05$, compared with the LPS + Gal-9 + anti-Tim-3 antibody group.

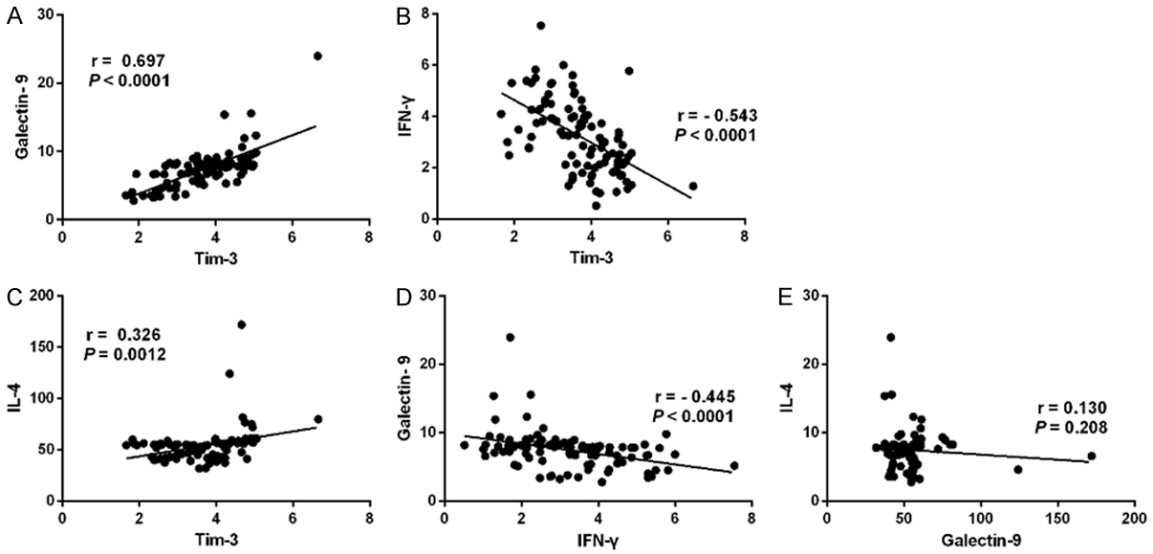

Figure 6. Association between Tim-3/Gal-9 with Th1 and Th2 cytokines.

The results showed that the expression of Tim-3^{+} T cells was elevated in sepsis, indicating that Tim-3 could be involved in the pathogenesis of sepsis.

It is widely known that Th1 cells can promote the effects of CTLs on cell proliferation, differentiation and killing ability. Th2 cells enhance the proliferation, differentiation and antibody production by B cells [6]. The pro-inflammatory cytokines secreted by Th1 cells, mainly IFN-γ, can eliminate the virus invading the host and enhance the antigen presentation process, whereas Th2 cells, characterized by the secretion of IL-4, suppress antigen presentation, thus resulting in T cell dysfunction [6]. Herein, an impaired Th1/Th2 cell balance was revealed in septic patients. However, the combination of Tim-3 with Gal-9 could attenuate the activation of Th1 cells, the production of IFN-γ, and the ability to clear pathogens, while it could enhance the body's tolerance to pathogens and promote immunosuppression [25, 26]. Therefore, in the present study lymphocytes were isolated from peripheral blood to establish an *in vitro* model simulating septic inflammation. The results demonstrated that the levels of IFN-γ were significantly increased in culture supernatants from LPS-stimulated lymphocytes. By contrast, the secretion of IFN-γ was reduced in cells treated with Gal-9 to activate Tim-3. Interestingly, no significant changes were observed in the secretory levels of IL-4. These results indicated that the combination of Tim-3 with Gal-9 could inhibit the activation of Th1 cells and the production of IFN-γ, thus regulating the Th1/Th2 balance during the inflammatory process of sepsis.

Subsequently, the association between Tim-3/Gal-9 and apoptosis was evaluated. Emerging evidence has suggested that the combination of Tim-3 with Gal-9 can promote T effector cell apoptosis, attenuate immune responses and induce immune tolerance [27, 28]. However, IFN-γ-secreted Th1 cells could increase the levels of Gal-9. The above process could be a part of the negative feedback mechanism of Tim-3$^+$ Th1 cell apoptosis [28, 29]. Therefore, the current study further investigated lymphocyte apoptosis. When the LPS-induced inflammatory model was established *in vitro*, the apoptosis rate of lymphocytes was significantly increased following cell treatment with Gal-9. By contrast, treatment of cells with anti-Tim-3 antibody notably reduced lymphocyte apoptosis. The aforementioned findings indicated that the binding of Gal-9 to Tim-3 could induce lymphocyte apoptosis.

In conclusion, the present study suggested that Tim-3, as a negative costimulatory molecule, could be involved in maintaining a balanced immune system. However, the current study has some limitations. For example, whether the combination of Tim-3 with Gal-9 could promote the apoptosis of T lymphocytes, particularly that of CD4$^+$ Th1 cells, was not investigated. Moreover, the effects of the Tim-3/Gal-9 axis on inflammation and clinical symptoms of patients with sepsis need to be further explored.

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Disclosure of conflict of interest

None.

Abbreviations

Tim-3, T cell immunoglobulin domain and mucin domain-3; sTim-3, soluble Tim-3; Th cells, T helper cells; TNF-α, tumor necrosis factor-α; WBC, white blood cells; CRP, C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; RBC, red blood cells; Hb, hemoglobin; PLT, Platelets.

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References

[1] Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G and Sccm/Esicm/Accp/Ats/Sis. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Crit Care Med 2003; 31: 1250-1256.

[2] Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, Angus DC and Reinhart K; International Forum of Acute Care Trialists. Assessment of global incidence and mortality of hospital-treated sepsis. Cur-

rent estimates and limitations. Am J Respir Crit Care Med 2016; 193: 259-272.
[3] Delano MJ and Ward PA. Sepsis-induced immune dysfunction: can immune therapies reduce mortality? J Clin Invest 2016; 126: 23-31.
[4] Ho MS, Mei SH and Stewart DJ. The immunomodulatory and therapeutic effects of mesenchymal stromal cells for acute lung injury and sepsis. J Cell Physiol 2015; 230: 2606-2617.
[5] Cabrera-Perez J, Condotta SA, Badovinac VP and Griffith TS. Impact of sepsis on CD4 T cell immunity. J Leukoc Biol 2014; 96: 767-777.
[6] Raphael I, Nalawade S, Eagar TN and Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 2015; 74: 5-17.
[7] Gupta DL, Bhoi S, Mohan T, Galwnkar S and Rao DN. Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with post traumatic sepsis. Cytokine 2016; 88: 214-221.
[8] Joller N and Kuchroo VK. Tim-3, Lag-3, and TIGIT. Curr Top Microbiol Immunol 2017; 410: 127-156.
[9] Asano T, Matsuoka N, Fujita Y, Matsumoto H, Temmoku J, Yashiro-Furuya M, Sato S, Suzuki E, Kobayashi H, Watanabe H and Migita K. Serum levels of T Cell immunoglobulin and mucin-domain containing molecule 3 in patients with systemic lupus erythematosus. J Clin Med 2020; 9: 3563.
[10] Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA, Freeman GJ and Kuchroo VK. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 2002; 415: 536-541.
[11] Tang F, Wang F, An L and Wang X. Upregulation of Tim-3 on CD4(+) T cells is associated with Th1/Th2 imbalance in patients with allergic asthma. Int J Clin Exp Med 2015; 8: 3809-3816.
[12] Dama P, Tang M, Fulton N, Kline J and Liu H. Gal9/Tim-3 expression level is higher in AML patients who fail chemotherapy. J Immunother Cancer 2019; 7: 175.
[13] Yasinska IM, Sakhnevych SS, Pavlova L, Teo Hansen Selno A, Teuscher Abeleira AM, Benlaouer O, Goncalves Silva I, Mosimann M, Varani L, Bardelli M, Hussain R, Siligardi G, Cholewa D, Berger SM, Gibbs BF, Ushkaryov YA, Fasler-Kan E, Klenova E and Sumbayev VV. The Tim-3-Galectin-9 pathway and its regulatory mechanisms in human breast cancer. Front Immunol 2019; 10: 1594.
[14] Anderson AC, Joller N and Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity 2016; 44: 989-1004.
[15] Yu J, Zhu R, Yu K, Wang Y, Ding Y, Zhong Y and Zeng Q. Galectin-9: a suppressor of atherosclerosis? Front Immunol 2020; 11: 604265.
[16] Li H, Wu K, Tao K, Chen L, Zheng Q, Lu X, Liu J, Shi L, Liu C, Wang G and Zou W. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. Hepatology 2012; 56: 1342-1351.
[17] Chabtini L, Mfarrej B, Mounayar M, Zhu B, Batal I, Dakle PJ, Smith BD, Boenisch O, Najafian N, Akiba H, Yagita H and Guleria I. TIM-3 regulates innate immune cells to induce fetomaternal tolerance. J Immunol 2013; 190: 88-96.
[18] Vendetti S, Riccomi A, Sacchi A, Sciaraffia E, Gatta L, Pioli C and De Magistris MT. Inhibition of T cell proliferation by cholera toxin involves the modulation of costimulatory molecules CTLA-4 and CD28. Immunol Lett 2008; 115: 59-69.
[19] Gao X, Zhu Y, Li G, Huang H, Zhang G, Wang F, Sun J, Yang Q, Zhang X and Lu B. TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. PLoS One 2012; 7: e30676.
[20] Bu M, Shen Y, Seeger WL, An S, Qi R, Sanderson JA and Cai Y. Ovarian carcinoma-infiltrating regulatory T cells were more potent suppressors of CD8(+) T cell inflammation than their peripheral counterparts, a function dependent on TIM3 expression. Tumour Biol 2016; 37: 3949-3956.
[21] Madireddi S, Eun SY, Lee SW, Nemcovicova I, Mehta AK, Zajonc DM, Nishi N, Niki T, Hirashima M and Croft M. Galectin-9 controls the therapeutic activity of 4-1BB-targeting antibodies. J Exp Med 2014; 211: 1433-1448.
[22] Johannes L, Jacob R and Leffler H. Galectins at a glance. J Cell Sci 2018; 131: jcs208884.
[23] Ungerer C, Quade-Lyssy P, Radeke HH, Henschler R, Konigs C, Kohl U, Seifried E and Schuttrumpf J. Galectin-9 is a suppressor of T and B cells and predicts the immune modulatory potential of mesenchymal stromal cell preparations. Stem Cells Dev 2014; 23: 755-766.
[24] Li X, Chen Y, Liu X, Zhang J, He X, Teng G and Yu D. Tim3/Gal9 interactions between T cells and monocytes result in an immunosuppressive feedback loop that inhibits Th1 responses in osteosarcoma patients. Int Immunopharmacol 2017; 44: 153-159.
[25] Cho JL, Roche MI, Sandall B, Brass AL, Seed B, Xavier RJ and Medoff BD. Enhanced Tim3 activity improves survival after influenza infection. J Immunol 2012; 189: 2879-2889.
[26] Mariat C, Sanchez-Fueyo A, Alexopoulos SP, Kenny J, Strom TB and Zheng XX. Regulation of

T cell dependent immune responses by TIM family members. Philos Trans R Soc Lond B Biol Sci 2005; 360: 1681-1685.

[27] Kang CW, Dutta A, Chang LY, Mahalingam J, Lin YC, Chiang JM, Hsu CY, Huang CT, Su WT, Chu YY and Lin CY. Apoptosis of tumor infiltrating effector TIM-3+CD8+ T cells in colon cancer. Sci Rep 2015; 5: 15659.

[28] Yang S, Wang J, Chen F, Liu G, Weng Z and Chen J. Elevated Galectin-9 suppresses Th1 effector function and induces apoptosis of activated CD4(+) T cells in osteoarthritis. Inflammation 2017; 40: 1062-1071

[29] Lee J, Park EJ, Noh JW, Hwang JW, Bae EK, Ahn JK, Koh EM and Cha HS. Underexpression of TIM-3 and blunted galectin-9-induced apoptosis of CD4+ T cells in rheumatoid arthritis. Inflammation 2012; 35: 633-637.