Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Dysregulated expression of T cell immunoglobulin and mucin domain 3 is associated with the disease severity and the outcome of patients with spontaneous intracerebral hemorrhage

Xiao Liu a,1, Junyu You a,b,1, Di Zhao a, Min Guo a, Yingfang Pan a, Lifen Gao a, Xiaohong Liang a,⁎, Chunhong Ma a,⁎

a Key Laboratory for Experimental Teratology of Ministry of Education and Institute of Immunology, Shandong University School of Medicine, 44 Wenhua Xi Road, Jinan, Shandong, 250012, PR China
b Laiwu Steel Group Hospital, Laiwu, Shandong 271126, PR China

A B S T R A C T

Objectives: We aimed to investigate the expression of T cell immunoglobulin and mucin domain 3 (Tim-3) on peripheral blood cells in spontaneous intracerebral hemorrhage (ICH) patients and to analyze its clinical significance.

Design and methods: Tim-3 expression on peripheral immunocytes from ICH patients and healthy volunteers was measured by flow cytometry. The correlation between Tim-3 expression and the clinical indices was estimated using linear regression.

Results: Tim-3 expression on peripheral CD3+ T cells and CD8+ T cells in ICH patients are significantly downregulated, while Tim-3 expressions on CD14+ monocytes and CD16+CD56+ NK cells are increased. Furthermore, Tim-3 expression on peripheral CD8+ T cells was negatively correlated with the inflammatory response, the disease severity and the outcome of ICH patients. However, there was no relationship between Tim-3 expression and blood glucose concentration.

Conclusions: Altered expression of Tim-3 might play an important role in the pathogenesis of ICH, demonstrating that Tim-3 might be a novel candidate molecule for prognosis evaluation of ICH patients.

© 2013 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

Introduction

Spontaneous intracerebral hemorrhage (ICH; nontraumatic and without any underlying lesion) is the most devastating type of stroke with limited effective therapies. It is characterized by spontaneous bleeding in the parenchymal tissue of the brain and is associated with a very high mortality and substantial morbidity [1]. Approximately 2 million cases of ICH occur worldwide each year [2], and patients with ICH have nearly twice the risk of being severely disabled when compared to patients with ischemic stroke [3]. Treatment for ICH is primarily supportive, and the clinical outcome is poor with potential huge burden for the caretakers. Better understanding of the pathogenesis of ICH-induced brain injury would contribute to improve the clinical outcome of ICH.

Increasing evidences from preclinical and clinical studies have described that an intense local inflammatory response surrounding the hemorrhage occurs soon after ICH and peaks several days later and have indicated that these inflammatory mechanisms contribute substantially to cell damage and edema formation caused by cerebral bleeding [4,5]. Napoli et al. reported that higher C-reactive protein (CRP), one of acute inflammatory markers, is associated with increased mortality in sICH patients and improved mortality prediction when added to the ICH score [6]. The inflammation cascades following ICH comprise both cellular components and molecular components [4]. Blood-derived leukocytes, macrophages, and resident microglia, that are activated and accumulate within the brain after ICH, are the major inflammatory cells. Animal models of ICH provide substantial evidences for the presence of leukocyte infiltration into the hematoma with the breakdown of the blood–brain-barrier (BBB) [7,8]. Clinical studies also support the role of leukocytes in ICH. Early studies by Molle [9] and Lee et al. [10] showed that leukocyte counts in cerebrospinal fluid were frequently elevated after ICH. Moreover, patients with ICH also have higher peripheral leukocyte counts [11], which were reported to be one of the independent predictors of neurologic deterioration in ICH [12]. Loftspring et al. [13] quantitatively detected the frequency of infiltrating leukocytes that enter the brain after ICH by using flow cytometry and found that, at 4 days ICH mice brain presented with a 3.4-fold increase in CD45+GR-1+ cells (mostly are neutrophils) and 1.7-fold increase in...
CD4+ T cells, compared with control mice, indicating that both innate and adaptive immune cells play roles in the development of brain injury after ICH.

Notably, immunoregulatory molecules are crucial for modulating the activation, proliferation and function of immunocytes in both physiological and pathological conditions, including in ICH. Toll-like receptor (TLR4), identified as an receptor for the recognition of pathogen-associated molecular patterns by immune cells, was found to be involved in the pathogenesis of ICH [14]. TLR4-deficient mice had markedly decreased perihematoma inflammation, associated with reduced recruitment of neutrophils and monocytes and improved functional outcome by day 3 after ICH. These results indicate that immunoregulatory molecules might be important mediators of immune damage ensuing ICH.

Tim-3, a member of T cell immunoglobulin and mucin domain family, was firstly identified as a specific cell surface marker on Th1 cells, but not on Th2 cells [15,16]. Further studies also described the abundant expression of Tim-3 on CD8+ T cells, monocytes/macrophages, mast cells, NK cells and dendritic cells [17–19]. A huge amount of data has disclosed the complicated functions of Tim-3 on different types of immune cells in the underlying physiological or pathological milieu [20]. Animal and clinical studies also proved that Tim-3 is involved in the pathogenesis of various kinds of diseases (e.g., tumor, viral infection, atherosclerosis, diabetes, and autoimmune diseases) by modulating the intensity and duration of innate and/or adaptive immune response [21,22]. More recently our research and that of several other groups suggested that Tim-3 might play a role in the inflammatory reaction in nervous system diseases. Zhang et al. [23] found that time course of Tim-3(+) cell accumulation correlated positively with disease progression of experimental autoimmune neuritis. Our previous findings showed that Tim-3 expression was upregulated in peripheral blood monocytes (PBMCs) of inflammatory stroke patients and correlated with abnormal lipid levels [24]. However, there is still no report about the roles of Tim-3 in the pathogenesis of ICH. Here, we found the different alterations of Tim-3 expression on PBMC subsets in ICH patients and, more importantly, there was significant correlation between Tim-3 expression on CD8+ T cells and the inflammatory response, the Glasgow coma scale score and the outcome of the patients. This work gives new insights into the inflammatory mechanisms of ICH and might provide a novel candidate molecule for prognosis evaluation and clinical treatment of ICH patients.

Materials and methods

Patients

We prospectively recruited all consenting patients admitted to Laiwu Steel Group Hospital (Laiwu, Shandong, China) with a diagnosis of ICH within 24 h after stroke onset between September 2010 and December 2011. Spontaneous ICH (sICH) was defined as sudden and spontaneous bleeding within the brain parenchyma confirmed by head CT scan, with or without intraventricular extension. Patients with hemorrhage secondary to trauma, intracranial tumor, hematological malignancy, and thrombolysis, or an underlying structural abnormality had been excluded by four senior neuroradiologists. To avoid other confounding factors, we excluded the patients with acute or chronic infections (≤2 months before sICH), those with autoimmune diseases or any other concurrent morbidities.

All patients were examined on hospital arrival. The Glasgow coma scale score (GCS), used to assess initial neurological deficit, was determined [25]. The related clinical data were collected: demographic data, blood pressure, and CT scan findings. The neuroradiologists, blinded to the clinical information, defined the site of ICH (basal ganglia, thalamic, lobar or other), volume of hematoma, and the presence of intraventricular hemorrhage. The baseline characteristics and potential clinical factors associated with 30-day mortality of the ICH patients were listed in Table 1. On arrival to the hospital, patients who died of ICH are younger (P < 0.05) and had lower Glasgow coma scale scores (P < 0.05), higher BG (P < 0.05), and higher WBC count (P < 0.05).

The control group consisted of 32 healthy volunteers from Medical Examination Center of Qilu Hospital, Jinan, Shandong. Exclusion criteria for controls were identical to those of ICH patients. The study was approved by the medical ethics committee of Shandong University, and an informed consent was acquired from each subject.

Measurement of blood markers

Blood glucose (BG) was determined by the Roche Diagnostics assay, HITACHI7600 automatic analyzer according to its protocol. White blood cell (WBC) counts were performed with flow cytometry.

Blood sampling and detection of Tim-3 expression on circulating immunocytes by flow cytometry

Blood samples were routinely taken from the antecubital vein from normal volunteers and ICH patients within 24 h since spontaneous hemorrhage onset. Flow cytometry was used to determine Tim-3 expression on peripheral blood immunocytes. One hundred microliters of whole blood was incubated at 4 °C in a dark room with monoclonal antibodies, FITC-conjugated anti-human CD4 (clone: OKT4; Bioscience, San Diego, CA), PE-conjugated anti-human CD3e (clone: UCHT1; ebioscience, San Diego, CA), PE-conjugated anti-Tim-3 (clone: RMT3-23; 2R&D, Minneapolis, MN), FITC-conjugated anti-CD16/56 (Serotec, Oxford, UK) and FITC-conjugated anti-CD14 (Jingmei Bio Tec, Shanghai, China). Thirty minutes later, stained blood samples were subjected to RBC lysis using a FACS lysis solution (BD Biosciences, San Jose, CA). Cells were washed once with a phosphate buffer solution (PBS) and were detected using a Beckman Coulter flow cytometer (Fullerton, CA, USA), and the data were analyzed using the Cell Quest program.

Outcome measures

The prognosis of ICH patients was estimated using Glasgow outcome scale (GOS) at 30 days. A good functional outcome was defined as GOS score 3 to 5 and a poor functional outcome was defined as GOS score 1 to 2.

Statistical analysis

All data were analyzed using the GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA). The Student’s t test and Mann–Whitney nonparametric U test were used for comparison between groups. Pearson correlation analysis was performed between the Tim-3 expression and blood glucose and white blood cell counts. P values were considered significant at P < 0.05.

Results

Dysregulated Tim3 expression on peripheral immunocytes in ICH patients

We first analyzed Tim3 expression on peripheral CD3+ T cells, CD4+ T cells, CD8+ T cells, CD14+ monocytes, and CD16+CD56+ NK cells in ICH patients and age- and sex-matched healthy controls. Flow cytometric analysis showed that Tim-3 expression on CD3+ T cells in patients with ICH was significantly lower than that of healthy controls (Fig. 1A,F, percentage of Tim3+ CD3+ T cells, ICH vs healthy, mean ± SD 4.5% ± 0.88% vs 6.4% ± 0.62%, P < 0.05); while Tim-3 expression on CD14+ monocytes (Fig. 1D,I) and CD16+CD56+ NK cells...
cells [Fig. 1E,J] were increased (percentage of Tim3+ CD14+ monocytes, ICH vs healthy, mean ± SD 90.1% ± 1.9% vs 79.8% ± 2.6%, P < 0.005; percentage of Tim3+ CD16+CD56+ NK cells, ICH vs healthy, mean ± SD 78.1% ± 2.4% vs 63.0% ± 3.1%, P < 0.005). Further analysis confirmed that Tim-3 expression on CD8+ T cells was decreased in ICH patients compared to healthy controls (Fig. 1C, percentage of Tim3+ CD8+ T cells, ICH vs healthy, mean ± SD 11.2% ± 3.2% vs 24% ± 2.6%, P < 0.005), while Tim-3 expression on CD4+ T cells had no change (Fig. 1B,G). These data indicated that Tim-3 expression on PBMC subsets was finely and differentially regulated at the early phase of ICH, which might suggest the complicated roles of Tim-3 and immunocytes in the pathogenesis of ICH.

Association between Tim-3 expression and disease severity of ICH patients on admission

Previous studies showed that inflammatory response contributes substantially to brain injury after ICH. Thus, we wonder whether the alteration of Tim-3 expression on immunocytes might also be involved in the pathogenesis of ICH. We analyzed the association of Tim-3 expression on CD8+ T cells, CD14+ monocytes, and CD16+CD56+ NK cells with the Glasgow coma scale score (GCS), which is one of the indicators for ICH. The statistical analysis showed that Tim-3 expression on CD8+ T cells was negatively correlated with the GCS score of ICH patients (Fig. 2B, Pearson r = −0.4671, P < 0.05). However, there were no significant associations between Tim-3 expressions on CD14+ monocytes or CD16+CD56+ NK cells with GCS score of ICH patients (Fig. 2C). These data clued that downregulated Tim-3 expression on CD8+ T cells might be a protective response at the early stage of ICH and alleviate the severity of brain injury.

Relationship between Tim-3 expression and the outcome of ICH patients

Considering that immune factors might be involved in deciding the prognosis and outcome of ICH [12], we then analyzed the relationship between Tim-3 expression and the GOS scores of ICH patients, one of the known indicators for ICH outcome. The statistical analysis showed that Tim-3 expression on CD8+ T cells was inversely correlated with the GOS scores of ICH patients (Fig. 3A, Pearson r = −0.5, P < 0.05). In contrast, there were no significant correlations between Tim-3 expressions on CD14+ monocytes or CD16+CD56+ NK cells with the GOS scores of ICH patients (Fig. 3B,C). Thus, these data demonstrate that altered Tim-3 expression on CD8+ T cells might be one of prognostic indicators for ICH.

Significance of altered Tim-3 expression on the inflammatory response of ICH patients

To further evaluate the significance of altered Tim-3 expression on the inflammatory response of ICH patients, we analyzed the relationship between Tim-3 expression on CD8+ T cells, CD14+ monocytes, or CD16+CD56+ NK cells and the white blood cell count in ICH patients. As shown in Fig. 4, Tim-3 expression on CD8+ T cells was positively correlated with the WBC count of ICH patients (Fig. 4A, Pearson r = 0.4520, P = 0.026). On the contrary, there was a negative association between Tim-3 expression on CD16+CD56+ NK cells and white blood cell count in ICH patients (Fig. 4C, Pearson r = −0.4149, P = 0.0245). However, no significant correlation was found between Tim-3 expression on CD14+ monocytes and white blood cell count in ICH patients (Fig. 4B). These results indicate that Tim-3 expression on immunocytes in ICH patients might modulate the ensuing inflammatory response, which in turn influences the severity and outcome of the patients.

Association of Tim-3 expression with blood glucose level

Owing to the importance of hyperglycemia both as an outcome determinant of ICH [27] and as a known regulator of inflammation [28], we then analyzed the association of altered Tim-3 expression of ICH patients with blood glucose level. The statistical analysis revealed that there were no significant correlations between blood glucose level and Tim-3 expression on CD8+ T cells, CD14+ monocytes, and CD16+CD56+ NK cells with blood glucose level. The statistical analysis showed that Tim-3 expression on CD8+ T cells was inversely correlated with the blood glucose level of ICH patients (Fig. 5, Pearson r = −0.4671, P < 0.05). These data clued that downregulated Tim-3 expression on CD8+ T cells might be a protective response at the early stage of ICH and alleviate the severity of brain injury.

Table 1

Baseline characteristics and potential clinical factors associated with 30-day mortality.

| Characteristics                                      | Total cohort n = 25 | Alive n = 15 | Death n = 10 | P      |
|------------------------------------------------------|---------------------|--------------|--------------|--------|
| Age, years (SD)                                      | 59.6 (11.8)         | 63.9 (9.4)   | 55.6 (12.5)  | 0.0389 |
| Male, n (%)                                          | 11 (47.8)           | 5 (45.5)     | 6 (54.5)     | 0.0941 |
| GCS score, median (IQR)                              | 8 (3–12)            | 11 (8–14)    | 3 (3–4)      | 0.00017 |
| GCS score, n (%)                                     |                     |              |              | 0.0016 |
| 13–15                                                | 6 (21.7)            | 6 (38.5)     | 0 (0)        |        |
| 9–12                                                 | 7 (30.4)            | 6 (46.2)     | 1 (10)       |        |
| 3–8                                                  | 12 (47.8)           | 5 (31.5)     | 8 (90)       |        |
| Biochemistry and vital signs on hospital arrival      |                     |              |              |        |
| BG, mmol/l, median (IQR)                             | 5.7 (5.0–11.5)      | 6.3 (5.3–8.5) | 12.15 (5.5–13.3) | 0.0463 |
| WBC, ×10⁷ mmol (median)                              | 9.6 (7.8–11.3)      | 8.7 (6.7–9.6) | 12.3 (10.4–16.6) | 0.0221 |
| SBP, mm Hg (SD)                                      | 170 (23)            | 167 (23)     | 173 (28)     | 0.3    |
| DBP, mm Hg (SD)                                      | 103 (14)            | 102 (16)     | 104 (13)     | 0.36   |
| Radiological variables                               |                     |              |              | 0.2512 |
| sICH localization, n (%)                             | 15 (60)             | 11 (73.3)    | 4 (40)       |        |
| Basal ganglia                                        | 4 (16)              | 1 (6.7)      | 3 (20)       |        |
| Thalamic                                             | 3 (12)              | 1 (6.7)      | 2 (20)       |        |
| Other                                                | 3 (12)              | 2 (13.4)     | 1 (10)       |        |
| Hematoma volume, mL, median (IQR)                    | 28 (24–70)          | 29 (24.8–70) | 25 (20–70)   | 0.4871 |
| IVH, n (%)                                           | 11 (44)             | 7 (46.7)     | 4 (40)       |        |
| Yes                                                  | 14 (56)             | 8 (53.3)     | 6 (60)       |        |

* P values of average or median values were analyzed by a non-parametric test.
* b P values of dispersion of different values were studied by Chi-square test.
Fig. 1. Dysregulated Tim3 expression on peripheral immunocytes in ICH patients. A–E show the percentage of Tim-3+ CD3+ T cells (A), Tim-3+ CD4+ T cells (B), Tim-3+ CD8+ T cells (C), Tim-3+ CD14+ monocytes (D), and Tim-3+ CD16+CD56+ NK cells (E) respectively. F–J show the representative plots of Tim-3 expression on CD3+ T cells (F), CD4+ T cells (G), CD8+ T cells (H), CD14+ monocytes (I), and CD16+CD56+ NK cells (J) respectively. Each dot represents one subject. Horizontal bars indicate the median Tim-3 percentage. P values are shown.
or CD16+CD56+ NK cells of ICH patients (Fig. 5). These results indicate that Tim-3 expression on immunocytes in ICH patients might be modulated in a glucose-independent manner.

**Discussion**

Both clinical and animal models of ICH proved that inflammatory response induces secondary brain injury leading to neuronal death, edema, and neurological disability [4,5], but the exact molecular mechanisms involved in this process are still not fully understood. Here, we carried out a pilot study about the potential role of Tim-3, a novel immunoregulatory molecule, in ICH. We found that Tim-3 expressions on peripheral CD8+ T cells, CD14+ monocytes, and CD16+CD56+ NK cells were significantly altered in ICH patients. Particularly, Tim-3 expression on CD8+ T cells has close relationships with the inflammatory response, the disease severity, and the outcome of ICH patients.

Innate immunocytes play important roles in inflammatory central nervous system (CNS) disorders, including ICH [29]. In the present study, we found that Tim-3 expressions in ICH patients were elevated on peripheral CD14+ monocytes and CD16+CD56+ NK cells (Fig. 1). Numerous studies have elucidated the regulatory roles of Tim-3, expressed on innate immune cells. Tim-3 expression on macrophages and dendritic cells promoted tissue inflammation by activating NF-κB and enhancing TNFα secretion [30]. Reported data about Tim-3 on NK cells are paradoxical. Gleason et al. demonstrated that Tim3 functioned as a receptor on NK cells to enhance IFN-γ production. Oppositely, our previous study showed that elevated Tim-3 expression on NK cells in chronic hepatitis B patients suppressed its cytotoxicity and IFN-γ secretion [31]. Unfortunately, we did not find any significant association of Tim-3 expression on peripheral CD14+ monocytes or CD16+CD56+ NK cells with the disease severity or the outcome of ICH patients (Fig. 2). It needs further investigation about the expression pattern of Tim-3 on infiltrating leukocytes in the brains of ICH patients and its dynamic change at the different stages of ICH.

Tim-3 also exerts key regulatory roles on adaptive immune cells and then participates in the pathogenesis of related inflammatory diseases. Interaction of Tim-3 and its ligand induced apoptosis of Th1 cells and inhibited Th1-mediated immunity [32]. Similarly, Tim-3 expression was related with the exhaustion of CD8+ T cells and ameliorated anti-tumor or anti-virus immunity [33]. Flow cytometry results showed that Tim-3 expressions on both CD3+ and CD8+ T cells were weakened in ICH patients (Fig. 1). More importantly, the statistical results revealed that the Tim-3 expression on CD8+ T cells was negatively correlated with GCS score on arrival (Fig. 2), 30-day GOS score (Fig. 3), and the white blood cell count (Fig. 5) of ICH patients. Similarly, Ndhlovu et al. [34] reported that patients with HTLV-1 associated myelopathy/tropical spastic paraparesis also had a systemic down-regulation of Tim-3 expression on virus-specific CD8+ T cells. Moreover, Tim3− CD8+ T cells showed highly active phenotype and might exert regulatory roles. Recently, the roles of CD8+ T cells in inflammatory CNS disorders have attracted particular attentions [35]. In autoimmune and infectious CNS diseases, brain-infiltrating CD8+ T cells exerted not only detrimental proinflammatory and killing functions but also regulatory function by direct killing of activated CD4+ T cells or by secretion of immunosuppressive cytokines such as IL-10 and transforming growth factor-β [36]. In mice with coronavirus-induced acute encephalitis, IL-10+ regulatory CD8+ T cells minimized immunopathological change and were more highly activated [37]. Our results and these reported data imply that the downregulated Tim-3 expression on CD8+ T cells might be probably a protective response in ICH patients and be helpful for patients’ recovery. However, the exact roles of CD8+ T cells and the effect of Tim-3 on CD8+ T cells in ICH are still needed to be further explored.

The changes of Tim-3 expression on immunocytes were induced by different inflammatory milieus. Thus, we preliminarily analyzed the potential mechanisms for altered Tim-3 expression in ICH patients. As shown in Table 1, blood glucose level in ICH patients was significantly higher than that of healthy controls, which is consistent with the reported data that hyperglycemia is one of risk factors for poor outcome of ICH [27,38]. More importantly, both clinical and
experimental data showed that acute or chronic hyperglycemia alters many cellular signaling pathways and is involved in the inflammatory processes [28]. Gonzalez et al. [39] showed that high glucose concentrations induce TNF-α production through the down-regulation of CD33. However, we found no significant association between blood glucose concentration and Tim-3 expression on CD8⁺ T cells, CD14⁺ monocytes or CD16⁺CD56⁺ NK cells in ICH patients. Till now, there are few reports about the underlying mechanisms leading to altered Tim-3 expression in pathological conditions. IL-12 and IL-4 were reported to be responsible for regulating Tim-3 expression in non-Hodgkin lymphoma [40] and in pregnancy [41] respectively. It is worthy to further study the role of other potential factors (e.g. cytokine profiles) in regulating Tim-3 expression in ICH patients.

Taken together, we report that an altered expression in the acute phase of human intracerebral hemorrhage and a significant correlation between Tim-3 expression on CD8⁺ T cells and the inflammatory response, the disease severity and the outcome of ICH patients, indicate that Tim-3 expression might become a novel candidate molecule for prognosis evaluation and clinical treatment of ICH patients.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

This study was supported in parts by Grants from the National Science Foundation of China (No. 91129704, No. 81171642 and No. 30901712), the Fok Ying Tung Education Foundation (No. 121038), the Foundation for Outstanding Young Scientist in Shandong Province (No. BS2011YY032) and the Independent Innovation Foundation of Shandong University, IIfSUD (No. 2011JC005).

References

[1] Broderick J, Connolly S, Feldmann E, Hanley D, Kase C, Krieger D, et al. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update: a guideline from the American Heart Association/American Stroke Association Stroke Council, High Blood Pressure Research Council, and the Quality of Care and Outcomes in Research Interdisciplinary Working Group. Stroke 2007;38:2001–23.
[2] Qureshi AI, Mendelow AD, Hanley DF. Intracerebral haemorrhage. Lancet 2009;373: 1652–44.
[3] Chiu D, Peterson L, Elkind MSV, Rosand J, Gerber LM, Silverstein MD, et al. Comparison of outcomes after intracerebral hemorrhage and ischemic stroke. J Stroke Cerebrovasc Dis 2010;19:225–9.
[4] Wang J, Dore’ S. Inflammation after intracerebral hemorrhage. J Cereb Blood Flow Metab 2007;27:894–908.
[5] Xi G, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral hemorrhage. Lancet Neurol 2006;5:53–63.
[6] Di Napoli M, Godoy BA, Campa V, del Valle M, Piñero G, Mirofsky M, et al. C-reactive protein level measurement improves mortality prediction when added to the spontaneous intracerebral hemorrhage score. Stroke 2011;42:1230–6.
[7] Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, et al. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 2006;12: 441–5.
[8] Wang J, Tiirku SE. Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. Brain 2005;128:1622–33.
[9] Molle WE. Leukocytosis in the cerebrospinal fluid in cerebral hemorrhage. Ohio Med J 1942;38:325–7.
[10] Lee MC, Heaney LM, Jacobson RL, Klassen AC. Cerebrospinal fluid in cerebral hemorrhage and infarction. Stroke 1975;6:638–41.
[11] Bestue-Cardiel M, Martin-Martinez J, Iturriaga-Heras C, Ara-Callizo JR, Oliveros-Juste A. Leukocytes and primary intracerebral hemorrhage. Rev Neurol 1999;29:368–71.
[12] Silva V, Leira R, Tejada J, Lainez JM, Castillo J, Davalos A. Molecular signatures of vascular injury are associated with early growth of intracerebral hemorrhage. Stroke 2005;36:86–91.
[13] Luftspring MC, McDole J, Lu A, Clark JF, Johnson AJ. Intracerebral hemorrhage leads to infiltration of several leukocyte populations with concomitant pathological changes. J Cereb Blood Flow Metab 2009;29:137–43.
[14] Sansing LH, Harris TH, Welsh FA, Kasner SE, Hunter CA, Kariko K. Toll-like receptor 4 contributes to poor outcome after intracerebral hemorrhage. Ann Neurol 2011;70: 646–56.
[15] Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, et al. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 2002;415:536–41.

Fig. 4. Significance of altered Tim-3 expression on the inflammatory response of ICH patients. The statistical results showed the association between white blood cell count and Tim-3 expression on CD8⁺ T cells (A), CD14⁺ monocytes (B), and CD16⁺CD56⁺ NK cells (C). Each dot represents a subject. r correlative coefficient and P values are shown.

Fig. 5. Association of Tim-3 expression with blood glucose level. The statistical results showed the association between blood glucose level and Tim-3 expression on CD8⁺ T cells (A), CD14⁺ monocytes (B), and CD16⁺CD56⁺ NK cells (C). Each dot represents a subject. r correlative coefficient and P values are shown.
Zhao D, Hou N, Cui M, Liu Y, Liang X, Zhuang X, et al. Increased T cell immunoglobulin and mucin-domain-containing molecule-3 (Tim-3) mediates natural killer cell suppression in chronic hepatitis B. J Hepatol 2010;52:322–9.

Sánchez-Fueyo A, Tian J, Picarella D, Domening C, Zheng X, Sabatos CA, et al. Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. Nat Immunol 2003;4:1093–101.

Sakushi K, Jayaraman P, Behar SM, Anderson AC, Kuchroo VK. Emerging Tim-3 functions in antimicrobial and tumor immunity. Trends Immunol 2011;32:345–9.

Ndhlovu LC, Leal FE, Hasenkrug JM, Jha AR, Carvalho KL, Eccles-James IG, et al. HTLV-1 tax specific CD8+ T cells express low levels of Tim-3 in HTLV-1 infection: implications for progression to neurological complications. PLoS Negl Trop Dis 2011;5:e1030.

Willing A, Friese MA. CD8-mediated inflammatory central nervous system disorders. Curr Opin Neurol 2012;25:316–21.

Smith TRF, Kumar V. Revival of CD8+ Treg-mediated suppression. Trends Immunol 2008;29:337–42.

Trandem K, Zhao J, Fleming E, Perlman S. Highly activated cytotoxic CD8 T cells express protective IL-10 at the peak of coronavirus-induced encephalitis. J Immunol 2011;186:3642–52.

Béjot Y, Abou-Eboulé C, Hervieu M, Jacquin A, Osseby GV, Rouaud O, et al. The deleterious effect of admission hyperglycemia on survival and functional outcome in patients with intracerebral hemorrhage. Stroke 2012;43:243–5.

González Y, Herrera MT, Soldevila C, García-García L, Fabián G, Pérez-Armendariz EM, et al. High glucose concentrations induce TNF-α production through the down-regulation of CD33 in primary human monocytes. BMC Immunol 2012;13:19.

Yang ZZ, Grote DM, Ziesmer SC, Niki T, Hirashima M, Novak AJ, et al. IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma. J Clin Invest 2012;122:1271–82.

Zhao J, Lei Z, Liu Y, Li R, Zhang L, Fang H, et al. Human pregnancy up-regulates Tim-3 in innate immune cells for systemic immunity. J Immunol 2009;182:6618–24.