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Gene expression changes in lymphocytes and monocytes from patients with traumatic brain injury

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Abstract

Background: Traumatic brain injury (TBI) can alter various immune functions, including immunosuppression, and constitutes a risk factor for nosocomial infections and organ dysfunction. Although TBI can induce a decline in immune cell function, the detailed mechanisms remains to be elucidated. We aim to explore transcriptional signatures associated with post-TBI immune alterations in a pilot cohort using a comprehensive transcriptome analysis of immune cells.

Methods: Three patients with traumatic brain injury and acute subdural hematoma were admitted to our hospital. We focused on three major subsets of immune cells responsible for the immune response: CD4+ T cells, CD8+ T cells, and monocytes. We evaluated the changes in immune function after injury using comprehensive transcriptome analysis. Blood samples were collected immediately after admission and one week later, and the data were compared with those of healthy volunteers.

Results: CD4+, CD8+ T cells, and monocytes, the expression of pathways involved in cellular metabolism—including oxidative phosphorylation,

mTORC1 signaling, MYC targets V1 and V2, and the unfolded protein response—was downregulated on day 7 compared with day 1. These findings suggest a transition from marked immune activation and metabolic upregulation on day 1 to an attenuated immune response by day 7. In CD4+ T cells, pathways associated with tissue remodeling and repair, such as hedgehog signaling and epithelial–mesenchymal transition, were upregulated from days 1 to 7, indicating a shift from inflammatory responses toward inflammation resolution and tissue repair.

Conclusions: In this pilot study, comprehensive transcriptome profiling suggests time-dependent transcriptional shifts in $CD4^+$ T cells, $CD8^+$ T cells, and monocytes after TBI. These findings should be interpreted as hypothesis-generating and provide a framework for larger, confirmatory studies.

Keywords: traumatic brain injury, lymphocyte, immunity

Background

In the United States, approximately 2.8 million individuals sustain traumatic brain injuries (TBIs) each year (1). Patients with severe TBI frequently develop nosocomial infections, including ventilator-associated pneumonia (2, 3). Therefore, clarifying the mechanism regulating the immune response in patients with TBI may help to improve treatment options. In TBI, central nervous system damage has been reported to influence peripheral immune responses through multiple pathophysiological mechanisms. Tissue injury caused by trauma leads to the release of various danger-associated molecular patterns and mediators, including ATP, HMGB1, and extracellular vesicles (4) (5) (6). This reaction is sterile, and these factors interact with immune cells to initiate an inflammatory response (7). In addition, injury to the central nervous system can induce systemic neurohumoral responses, such as catecholamine release, which act on peripheral organs including the spleen and liver, thereby modulating T-cell activation and immune function (8) (9). This inflammatory response is known as systemic inflammatory response syndrome (SIRS) and is defined as an excessive systemic inflammatory response exhibited by the host in response to various insults, including infection, trauma, and ischemia (10). A compensatory anti-inflammatory response syndrome (CARS) has also been described to occur simultaneously in response to trauma (11). This concept, proposed by Bone et al. (12), describes an adaptive host response in which immune activity is suppressed in reaction to excessive inflammatory stimuli. When CARS becomes excessive or prolonged, it may result in immunoparalysis, thereby increasing the risk of secondary infections and organ dysfunction (13). T cells play a major role in the modulation of immunosuppressive mechanisms. In particular, the reduction in CD4+ T cells and the increase in PD-1 expression in CD8+ T cells reflect immune fatigue (14). In trauma patients, the proportions of CD4+ and CD8+ T cells have been reported to decline during the first week after injury and, in cases

complicated by SIRS, these reductions often fail to recover even after 1 week (15) (16). This has been explained by the fact that the compensatory host reaction to strong inflammation is most clearly observed approximately one week after injury (17). Aside from T cells, monocytes and macrophages mediate immunosuppression, and a trauma-induced inflammasome dysfunction is possibly related to immunosuppression (13, 18, 19) .

Conventionally, immunosuppression has been evaluated based on changes in the number of immune cells and the amount of cytokine production (15). However, the mechanisms and detailed pathology of the decline in functional immune cells remains to be characterized. Recent advances in genome analysis have facilitated comprehensive transcriptome analysis (20), whereby trauma-induced changes in RNA expression have been identified (21, 22). Comprehensive transcriptome analysis can potentially provide novel insight into immunosuppressive mechanisms that could not previously be captured by studies focusing exclusively on cytokines or specific genes. Furthermore, functional changes can be monitored even when there is no change in cell numbers. The characterization of the underlying mechanisms and functional changes associated with immunosuppression may lead to treatments capable of preventing it and thus inhibiting secondary infections. However, few transcriptome analyses of the response to TBI have been reported, and there are no available studies on the immune function of patients affected by TBI.

We conducted a small exploratory pilot study examining time-dependent changes in three immune cell populations involved in immune function ($CD4^+$ T cells, $CD8^+$ T cells, and monocytes) during the first week after admission, when immunosuppression is thought to occur.

Methods

Study design and participants

We conducted a prospective, single-center, exploratory pilot study with a case–control framework. The study protocol was approved by the Institutional Review Board of the University of Osaka hospital (approval number: 885) and complied with the principles of the Declaration of Helsinki. Written informed consent for the collection of blood samples and the use of clinical data was obtained from the patients or their relatives, and from healthy volunteers.

In this study, we included patients with isolated TBI that were admitted to our hospital between March 2022 and July 2022 and required emergency neurosurgical surgery for acute subdural hematoma. We defined patients with isolated TBI as those who only presented an injury with an Abbreviated Injury Scale 98 (AIS-98) score equal or above 3 in the head and an AIS-98 score below 3 in areas other than the head. TBI encompasses a wide spectrum of injury severity and hematoma morphology; therefore, its pathological features are expected to vary considerably. To minimize patient heterogeneity as much as possible, we focused on isolated head injury and included only patients with a uniform hematoma type, namely acute subdural hematoma, which is known to be associated with severe injury and high mortality (23). In addition, we restricted the study population to patients who underwent neurosurgical intervention to ensure a comparable degree of treatment-related invasiveness. Accordingly, the inclusion criteria were the presence of acute subdural hematoma and the requirement for neurosurgical surgery. The exclusion criteria were death within 7 days after admission and the use of immunosuppressive agents before admission or within the first 7 days of hospitalization. The control population consisted of volunteers enrolled via public poster advertisements.

Sample collection and clinical data

Samples from the patients were collected at two time points: within 24 hours of admission and on the seventh day after admission (Figure 1a). Blood samples were stored at −80 °C until analysis. The clinical data collected by the investigators from the electronic medical records of the patients included age, sex, Glasgow Coma Scale (GCS) score at admission, site and AIS of whole-body trauma, Injury Severity Score (ISS), type of neurosurgical surgery, presence or absence of tracheotomy, duration of mechanical ventilation, presence or absence of nosocomial infection, and Glasgow Outcome Scale (GOS) score at discharge. The following blood parameters were determined from blood samples collected immediately after admission: fibrinogen, prothrombin time-international normalized ratio, activated partial thromboplastin time, fibrinogen degradation products, and D-dimer. Pneumonia, a nosocomial infection, was diagnosed as hospital-acquired pneumonia based on standard clinical and radiological imaging diagnoses (24).

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh whole blood collected in heparin-coated tubes by density gradient centrifugation using Leucosep (Greiner Bio-One, Kremsmünster, Austria) according to the manufacturer's instructions. Isolated PBMCs were divided in half and stored in CELLBANKER cell freezing medium (Nippon Zenyaku Kogyo Co. Ltd., Fukushima, Japan) at −80 °C until use (25). One half was sorted using fluorescence-activated cell sorting, and the number of cells in each subset was quantified. The other half was sorted into subsets, and RNA sequencing was performed (Figure 1b).

Fluorescence-activated cell sorting

Two panels were used to identify subsets of CD4+ T cells, CD8+ T cells, and monocytes from PBMCs. First, DAPI was used to extract viable cells. Lymphocytes and CD3+ cells were extracted. Positive subsets for the CD4 and CD8 markers were defined as CD4+ and CD8+ T cells. Monocytes were extracted from the second panel. DAPI was used for the extraction of viable cells.

RNA sequencing and bioinformatics

Total RNA was extracted from the sorted cells using QIAzol lysis reagent (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The RNA quantity and integrity were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc, Santa Clara, CA). RNA sequencing (RNA-seq) libraries were prepared using a TruSeq stranded mRNA sample preparation kit (Illumina, San Diego, CA) following the manufacturer's instructions. Whole-transcriptome sequencing was performed on an Illumina NovaSeq 6000 platform using 101-base pair pair-end reads. Sequencing reads were aligned to the human reference genome (hg19) using TopHat (version 2.1.1). Gene expression was quantified as fragments per kilobase of exon per million mapped fragments using Cufflinks (version 2.2.1). Differential gene expression analysis was performed by comparing samples obtained within 24 hours of admission with those obtained on hospitalization days 7–8 using the edgeR package (version 3.19) in R (26), based on normalized read counts. Analyses were conducted separately for each immune cell subset (CD4+ T cells, CD8+ T cells, and monocytes) using samples from three

patients (Patients 1–3) and healthy controls. Hierarchical clustering analysis was conducted to assess global similarities and differences in gene expression profiles among the samples. Gene Set Enrichment Analysis (GSEA) was performed using the curated hallmark gene sets from MSigDB (27, 28).

Statistical analysis

Summary data are presented as median (interquartile range [IQR]) for continuous variables and as number (%) for categorical variables. The Mann-Whitney U test was used to evaluate differences between the two groups for continuous variables, and the chi-squared test and Fisher's exact test were used for dichotomous variables. Statistical analysis was performed using commercially available statistical analysis software (JMP pro 16 software, SAS Institute Inc., Cary, NC, USA). A p-value < 0.05 was considered statistically significant.

ELISA

Serum samples prepared during PBMC isolation and stored at −30 °C were used for cytokine measurements. Plasma concentrations of interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-27 (IL-27), and interferon-γ (IFN-γ) were quantified using enzyme-linked immunosorbent assays (ELISA; R&D Systems, Minneapolis, MN).

Frozen serum samples were thawed and analyzed according to the manufacturer’s protocols. Absorbance was measured using a microplate reader (SH-9000Lab; Corona Electric Co., Ltd., Ibaraki, Japan).

Results

Participant characteristics

Between March 2022 and July 2022, 17 patients with isolated TBI were admitted to our hospital. Of these, three required emergency neural surgery for acute subdural hematoma. No patient died within 7 days of admission. Two healthy volunteers participated in the study. The backgrounds of the three patients and two healthy volunteers included in the analysis are shown in Table 1. The patients ages were 62, 78, and 76 years, and two of them were men. The types of neural surgery were as follows: craniotomy in two cases, and craniectomy in one case. Tracheotomy and nosocomial infection (pneumonia) occurred in three cases. The GOS at discharge were 3, 2, and 2 (Table 1). The two healthy individuals were a 49- and a 60-year-old man with no underlying or ongoing disease. Samples from the first three consecutive cases with TBI (Pt. 1, 2, and 3) were used to retrieve CD4+ T cells, CD8+ T cells, and monocytes.

Fluorescence-activated cell sorting results

The proportions of CD4+ T cells, CD8+ T cells, and CD14+ monocytes among total viable cells were calculated for samples obtained from Patients 1–3 on day 1 and day 7 after injury, as well as from healthy controls. The proportion of CD4+ T cells among the total viable cells obtained from patients was 25.7% within 24 hours of admission, which was similar to that obtained from healthy subjects (27.0%). However, this proportion decreased to 18.4% on days 7–8. In the CD8+ T cell subset, the percentage was 7.0% within 24 hours of hospitalization and 4.6% on days 7–8,

whereas the proportion in healthy volunteers was 17.4%. In the monocyte subset, the percentage was 3.2% in healthy individuals, 25.0% within 24 hours of hospitalization, and 23.7% on days 7–8 (Table 2). In the day 8 sample from Patient 1, cell recovery was insufficient, resulting in a markedly low proportion relative to the total number of viable cells. Because an adequate number of cells could not be obtained, RNA sequencing could not be reliably performed, and this sample was therefore excluded from the transcriptome analysis.

Differences in transcriptome analysis for each fraction

When dendrograms were generated, CD4+ T cells from patients with TBI (Patients 1, 2, and 3) on day 1 clustered closely with those from healthy controls, showing a relatively small Euclidean distance, whereas samples obtained from patients on day 7 were more distantly separated. Similar clustering patterns were observed for CD8+ T cells and monocytes (Figure2).

Next, GSEA was performed on RNA sequencing data from CD4+ T cells by comparing samples obtained from patients on day 1 and day 7 after injury; given the limited sample size, the results were interpreted descriptively to identify dominant transcriptional patterns rather than to draw definitive conclusions (Figure 3). Pathways related to oxidative phosphorylation, mtorc1 signaling, MYC targets V1 and V2, and the unfolded protein response were identified among the top enriched pathways. All these pathways exhibited negative normalized enrichment scores, indicating downregulation on day 7 compared with day 1. Many additional pathways were also downregulated (Table S1).

Similar GSEA analyses comparing days 1 and 7 samples were performed for CD8+ T cells and monocytes, revealing enrichment of pathways largely overlapping with those identified in CD4+ T cells (Table S2). In CD4+ T cells and monocytes, pathways associated with cell cycle regulation, including E2F targets and G2M checkpoint pathways, were also downregulated on day 7 compared with day 1 (Table S3).

In contrast, pathways that were upregulated on day 7 relative to day 1 were observed specifically in CD4+ T cells and included hedgehog signaling and epithelial–mesenchymal transition (EMT).

Finally, leading-edge analyses were conducted for each enriched pathway. For oxidative phosphorylation, AIFM1 was identified as a leading-edge gene in CD4+ T cells, COX6C in CD8+ T cells, and ATP6V1F in monocytes. For MYC targets V1, the leading-edge genes were XRCC6 in CD4+ T cells, PRLP0 in CD8+ T cells, and PSMD14 in monocytes (Tables S1–S3).

To further visualize transcriptional shifts across immune cell subsets, we generated a heatmap of leading-edge gene expression across CD4+ T cells, CD8+ T cells, and monocytes. The heatmap illustrates distinct, cell type–specific expression patterns of leading-edge genes associated with oxidative phosphorylation, mTORC1 signaling, MYC targets, hedgehog signaling, and EMT. Consistent with the GSEA results, genes related to metabolic and cell cycle pathways showed broadly reduced expression on day 7 compared with day 1 across multiple immune cell types, whereas hedgehog signaling and EMT-associated genes demonstrated selective upregulation in CD4+ T cells (Figure 4).

ELISA

Serum samples obtained from three patients with TBI and two healthy controls were analyzed, as shown in Figure S1. Although statistical evaluation was limited due to the small sample size, IL-6 levels tended to be low in healthy controls and on day 1 after injury, followed by an increase toward day 7.

In contrast, levels of IL-10, IL-27, and IFN-γ were lower on day 1 in patients TBI compared with healthy controls, but showed a tendency to

increase toward levels comparable to those of healthy controls by day 7 (Figure S1).

Discussion

In this study, we evaluated changes in gene expression profiles of CD4+ T cells, CD8+ T cells, and monocytes in patients with TBI by comparing samples obtained from healthy controls and from patients on days 1 and 7 after injury. Hierarchical clustering analysis revealed that, in all three cell populations, gene expression profiles from healthy controls clustered closely with those from patients on day 1, whereas samples from patients on day 7 formed distinct clusters separated from both groups. These findings suggest that, while physiological immune responses are evident on day 1 after injury, progressive alterations in gene expression occur between day 1 and day 7, leading to pathological immune dysfunction that differs from the immune state observed in healthy individuals.

GSEA identified pathways exhibiting dynamic changes in gene expression from day 1 to day 7. In CD4+ T cells, CD8+ T cells, and monocytes, pathways related to oxidative phosphorylation, mTORC1 signaling, and MYC targets V1 and V2 were consistently downregulated by day 7, indicating transcriptional suppression at this time point and, conversely, upregulation on day 1. Upregulation of oxidative phosphorylation reflects increased ATP production and enhanced mitochondrial activity, indicating that immune cells are placed in a highly energy-demanding state (29, 30). Activation of mTORC1 signaling and MYC target pathways promotes glucose uptake, mRNA translation, and amino acid utilization, enabling immune cells to acquire the “biosynthetic capacity” required for effector functions and cytokine production (31, 32). These findings suggest metabolic reprogramming that supports immune cell differentiation into effector cells.

In the acute phase of TBI, immune cells appear to exhibit enhanced intracellular metabolism and activated proliferative signaling, indicating broad mobilization of immune cell populations in response to inflammation. Notably, in CD4+ T cells and monocytes, pathways related to cell cycle regulation, including E2F targets and G2M checkpoint pathways, were also upregulated on day 1, supporting the notion that immune cells enter a proliferative state during the acute phase (33). Thus, immune responses following TBI involve not only cellular activation but also increases in cell number and differentiation. By day 7, however, in addition to suppression of metabolic and proliferative pathways, pathways associated with DNA repair, unfolded protein response, and interferon signaling—reflecting DNA damage, endoplasmic reticulum stress, and immune activation—were also downregulated (34) (35, 36). These findings indicate a resolution of immune activation and a transition toward an immunosuppressive phase accompanied by pathological functional decline. Such changes are notable because they may be associated with the development of T cell exhaustion and immunometabolic paralysis (37, 38).

When analyzed by cell population, CD4+ T cells exhibited not only attenuation of immune activation but also upregulation of pathways related to tissue remodeling and repair, including hedgehog signaling and epithelial–mesenchymal transition (EMT), from day 1 to day 7 (39, 40). This pattern suggests a transition from acute inflammatory responses toward tissue repair and features characteristic of the chronic inflammatory phase. CD4+ T cells are generally responsible for cytokine production and the activation of CD8+ T cells (41, 42). Suppression of CD4+ T cell metabolism and proliferation may therefore impair regulation of other immune cell populations, potentially contributing to immunoparalysis. In the present study, the proportion of CD4+ T cells did not differ substantially from that of healthy controls on day 1 but decreased by day 7, suggesting that convergence of transcriptional suppression and numerical decline may lead to impaired immune function. CD8+ T cells play

critical roles in amplifying innate immunity through IFN-γ production, defending against infection, and mediating cytotoxic immune responses (43). In TBI, CD8+ T cells may undergo acute activation in response to excessive antigenic stimulation and inflammatory environments, followed by marked functional decline and metabolic suppression by day 7. This pattern is consistent with immunoparalysis models described in sepsis, including T cell exhaustion and metabolic paralysis, and may help explain the increased risk of nosocomial infection and poor outcomes after traumatic brain injury (44).

Monocytes serve as central mediators of innate immune responses in the acute phase of traumatic brain injury, contributing to tissue factor release and induction of inflammatory cytokines, as well as functioning as antigen-presenting cells that activate CD4+ and CD8+ T cells (45). Although the proportion of monocytes was higher in patients with traumatic brain injury than in healthy controls on both day 1 and day 7, GSEA suggested a decline in metabolic activity from days 1 to 7. This likely resulted in reduced cytokine production and impaired antigen presentation to other immune cells, representing functional changes not captured by cell counts alone.

In addition, circulating cytokine levels were measured to complement the transcriptomic assessment of immune function after traumatic brain injury. IL-6 levels were elevated on day 1 compared with healthy controls and increased further by day 7, reflecting sustained systemic inflammation following trauma. Although statistical analysis was limited by sample size, the anti-inflammatory and immunoregulatory cytokines IL-10 and IL-27 (46, 47) were reduced on day 1 and partially recovered toward healthy control levels by day 7, suggesting progression of anti-inflammatory responses during hospitalization. IFN-γ, which is produced by Th1 cells and $CD8^+$ T cells and plays a key role in immune defense, was also reduced on day 1 and partially recovered by day 7. This pattern suggests that immune cell function is acutely suppressed

immediately after injury, with gradual recovery over time. Together, these cytokine profiles indicate an initial inflammatory surge accompanied by suppression of cellular immunity, followed by persistent inflammation and progression of anti-inflammatory responses. These findings are consistent with the transcriptomic evidence of acute immune activation followed by immune suppression in $CD4^+$/$CD8^+$ T cells and monocytes.

In summary, all three immune cell populations—$CD4^+$ T cells, $CD8^+$ T cells, and monocytes—exhibited a transition from strong immune activation and metabolic upregulation on day 1 to a hyporesponsive immune state by day 7. Distinct cell-specific patterns were observed: $CD4^+$ T cells demonstrated a shift toward inflammation control and tissue repair, $CD8^+$ T cells showed features suggestive of exhausted cytotoxic function, and monocytes exhibited suppression of innate immune activity. These temporal immune alterations may influence susceptibility to infection; however, this hypothesis requires validation in larger, adequately powered cohorts.

TBI affect peripheral immune responses due to brain damage, and it has been suggested that this may increase the risk of secondary damage to distant organs as well as that of infection. (13) All three of the cases analyzed in this study developed pneumonia, and secondary infections occurred during the course of the disease. The cells responsible for immune protection against infection, including CD4+ T cells, CD8+ T cells, and monocytes, were unable to maintain their function by day 7, preventing the effective control of the infection.

Although the results of this study do not represent a major change in current trauma treatment strategies, they provide preliminary information that may help inform future investigations into immune dysfunction following TBI. In this study, the comparison was made with healthy individuals, not with patients affected by trauma but without a specific traumatic brain injury. Therefore, the results may not necessarily reflect changes caused by the brain injury itself, but rather by the general effect of suffering trauma. If the effects of brain injury are to be evaluated,

it is necessary to make a comparison with cases affected by trauma but without a brain injury. In this study, we were able to capture broad changes occurring in CD4+ and CD8+ T cells. Each of them can be classified into many important subsets, such as regulatory T cells. Although discriminating between these subsets is important to characterize immune function in detail, this was not possible with our experimental setting because we could not isolate a sufficient number of cells for analysis from the blood samples. This limitation may be overcome by using new technologies such as single-cell sequencing. The results of this study may provide a platform for further elucidation of the pathology and treatment of patients with TBI.

Limitations

This study has some limitations inherent to its exploratory pilot design. Accordingly, all findings should be interpreted as hypothesis-generating rather than confirmatory. As this was a single-center study, the patient sample size was small and the analysis was exploratory. Additionally, the control group consisted of only two individuals. Comprehensive transcriptome analysis based on RNA sequencing involves thousands of genes, and since it is not possible to predefine which variables should be used to estimate statistical power, accurate estimation of the expected effect size was difficult. Furthermore, because this study only evaluated changes in patients with TBI, the results obtained cannot be considered TBI-specific. To minimize heterogeneity, we focused on patients with acute subdural hematoma that required neurosurgical intervention. Different results may be obtained for other types of TBI, such as acute epidural hematoma or diffuse axonal injury. Since the first blood sample was collected within 24 hours of admission, the condition of individual patients may have showed variations depending on the exact number of hours

elapsed between injury and sample collection. In addition, follow-up samples from healthy controls at 1 week were not evaluated; therefore, the potential impact of temporal variation cannot be completely excluded. Nevertheless, it is unlikely that pathological changes in immune function would occur within 1 week in healthy individuals. Furthermore, the information obtained from transcriptome analysis only evaluates RNA expression at the time the sample was taken, and it is not possible to conclude whether it indicates the cause or result of the condition. In this study, RNA was extracted using the QIAzol method, and some samples exhibited low RINs (Table S4). Although samples with low RIN values may have influenced the results, the QIAzol method is a widely used standard extraction technique, and this limitation should be interpreted within the context of the study design.

Conclusions

This exploratory pilot transcriptome analysis suggests time-dependent transcriptional changes in $CD4^+$ T cells, $CD8^+$ T cells, and monocytes during the first week after TBI, when immunosuppression is thought to occur. These preliminary findings provide human data that may inform future large-scale, cell-specific immunological studies in TBI.

List of abbreviations

AIS-98, Abbreviated Injury Scale 98; CARS, compensatory anti-inflammatory response syndrome; GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale; HLA, human leukocyte antigen; IQR, interquartile range; ISS, Injury Severity Score; PBMC, peripheral blood mononuclear cell; SIRS, systemic inflammatory response syndrome; TBI, traumatic brain injury

Declarations

Ethics approval and consent to participate: The study protocol was approved by the Institutional Review Board of the University of Osaka hospital (Approval Number: 885) and complied with the principles of the Declaration of Helsinki. Written informed consent for the collection of blood samples and the use of clinical data was obtained from the patients or their relatives, and from healthy volunteers.

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article. The raw data from this study were submitted to the Gene Expression Omnibus under accession numbers GSE285385 and GSE314947.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: HI, MI, HM, HO, and DO were involved in the conception and design of the study. HI performed the literature review. HI and MI acquired the data and performed the analysis and interpretation. HI drafted the manuscript. HI, MI, HM, HO, and DO were involved in the critical revision of the manuscript. All authors contributed to the discussions, managed the study, and read and approved the final version of the manuscript.

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Figure legends

Figure 1. Workflow of our research

(a) Samples from the control population and the patients were collected. (b) One half of PBMCs was sorted using FACS, and the number of cells in each subset was quantified. The other half was sorted into subsets, and RNA sequencing was performed.

PBMCs, Peripheral blood mononuclear cells; FACS, fluorescence-activated cell sorting.

Figure 2. Hierarchical clustering analysis of gene expression in patients with traumatic brain injury and healthy controls

Dendrograms illustrating RNA expression profiles of CD4+ T cells, CD8+ T cells, and monocytes obtained from patients with traumatic brain injury (Patients 1, 2, and 3) on day 1 and day 7 after injury, as well as from healthy controls.

Figure 3. Gene Set Enrichment Analysis (GSEA) of hallmark pathways in immune cell subsets comparing day 7 versus day 1

This dot plot illustrates the significant Hallmark pathways enriched in CD4, CD8, and CD14 cells at Day 7 compared to Day 1. The pathways represent the union of the top 5 upregulated and top 5 downregulated gene sets ranked by Normalized Enrichment Score (NES). A positive NES (red) indicates upregulation in Day 7, while a negative NES (blue) indicates downregulation relative to Day 1. The dot size is proportional to the statistical significance (−log10 adjusted p-value).

Figure 4. Heatmap of leading-edge gene expression across immune cell subsets

This heatmap displays the relative expression levels of leading-edge genes associated with four key hallmark pathways: Oxidative phosphorylation, mTORC1 signaling, hedgehog signaling, and epithelial mesenchymal transition (EMT). Columns are organized by cell type (CD4, CD8, CD14) and time point (day 1 vs day 7). Expression values (TPM) were log2-transformed and row-scaled (Z-score). Red indicates upregulation and blue indicates downregulation relative to the mean expression of each gene.

Table 1. Clinicodemographic characteristics of the participants

Characteristics	Patient No.			Healthy control No.	
	1	2	3	1	2
Age, years	62	78	76	49	60
Sex	Female	Male	Male	-	-
Admission GCS	3	3	6	-	-
AIS-head	5	5	5	-	-
ISS	29	25	25	-	-
Fibrinogen*, mg/dL	261	361	249	-	-
PT-INR*	1.11	1.08	1.03	-	-
aPTT*, s	30	26	27	-	-
FDP*, μg/mL	262.5	4.4	69.9	-	-
D-dimer*, μg/mL	68.25	2.21	20.61	-	-
Types of neurosurgical procedures	Craniotomy	Craniotomy	Craniectomy	-	-
Tracheostomy	+	+	+	-	-
Blood products				-	-
Fresh Frozen Plasma, units	14	4	10	-	-
Red Cell Concentrate, units	8	4	2	-	-
Platelet Concentrate, units	20	0	0	-	-
Duration of ventilator use, days	13	7	9	-	-
Types of nosocomial	Pneumonia	Pneumonia	Pneumonia	-	-
Immunomodulatory treatment before admission	-	-	-	-	-
Immunomodulatory treatment after admission	-	-	-	-	-
GOS at discharge	3	2	2	-	-

AIS: Abbreviated Injury Scale, aPTT: activated partial thromboplastin time, FDP: fibrinogen degradation products, GOS: Glasgow Outcome Scale, GCS: Glasgow Coma Scale, IQR: interquartile range, ISS: Injury Severity Score, PT-INR: prothrombin time-international normalized ratio
*Parameters reported from blood sample collected at admission.

Table 2. Ratio to live cells per subset (%)

Subset		**Control**	**Within 24 hours after admission**	**7–8 days after admission**
lymphocyte	CD4+	27.0	25.7	18.4
	CD8+	17.4	7.0	4.6
myeloid	monocyte	3.2	25.0	23.7