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IP-10, a Potential Driver of Neurally-Controlled IL-10 and Morbidity in Human Blunt Trauma

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Abstract

Objective—Blunt trauma and traumatic spinal cord injury (TSCI) induce systemic inflammation that contributes to morbidity. Dysregulated neural control of systemic inflammation post-injury is likely exaggerated in patients with TSCI. We utilized *in silico* methods to discern dynamic inflammatory networks that could distinguish systemic inflammation in TSCI from blunt trauma.

Study Design—Retrospective study

Settings—Tertiary care institution

Patients—21 severely-injured thoraco-cervical TSCI patients and matched 21 severely-injured blunt trauma patients without spinal cord injury (non-SCI).

Intervention—None

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Measurements and main results—Serial blood samples were obtained from day 1–14 post-injury. Twenty-four plasma inflammatory mediators were quantified. Statistical significance between the two groups was determined by Two-way ANOVA. Dynamic Bayesian Network inference (DBN) was used to suggest dynamic connectivity and central inflammatory mediators. Circulating IL-10 was significantly elevated in thoraco-cervical TSCI vs. non-SCI, whereas IL-1 β , sIL-2R α , IL-4, IL-5, IL-7, IL-13, IL-17, MIP-1 α and -1 β , GM-CSF, and IFN- γ were significantly reduced in TSCI vs. non-SCI. DBN suggested that post-SCI IL-10 is driven by IP-10, whereas MCP-1 was central in non-SCI dynamic networks. In a separate validation cohorts of 356 non-SCI patients and 85 TSCI patients, individuals with plasma IP-10 levels $\geq$ 730 pg/ml had significantly prolonged hospital and ICU stay and days on mechanical ventilator vs. patients with plasma IP-10 < 730 pg/ml.

Conclusion—This is the first study to compare the dynamic systemic inflammatory responses of TSCI vs. non-SCI patients, suggesting a key role for IP-10 in driving systemic IL-10 and morbidity and highlighting the potential utility of *in silico* tools to identify key inflammatory drivers.

Keywords

Chemokines; Spinal Cord Injuries; Trauma; Nervous System; Inflammation; Neurogenic Inflammation

INTRODUCTION

Blunt trauma, is a leading cause of death worldwide among young people (1). Traumatic spinal cord injury (TSCI) disproportionately affects young, healthy individuals (2, 3), with a 10-year mortality rate of ~16% (4). Both TSCI and blunt trauma without SCI trigger a systemic inflammatory response syndrome (SIRS) (5, 6) that precipitates organ failure (5) and contributes to secondary infection (7).

The complex interplay between local and systemic inflammation and the association of dysregulated systemic inflammation with adverse outcomes post-trauma (with or without concomitant TSCI) have stymied the search for novel therapies. We have previously suggested that computational modeling focused on defining mechanisms from dynamic patterns (8–11) may be required to extract translationally-relevant information from the increasing wealth of high-dimensional clinical (12) and molecular (13) data being generated in the setting of human traumatic injury.

Accordingly, we sought to utilize emerging *in silico* methods for analysis of dynamic biological networks in order to gain insights regarding central drivers and networked modules that differentiate the systemic inflammatory response to blunt trauma vs. TSCI. We show for the first time that the dynamic, systemic inflammatory responses of TSCI patients are altered significantly relative to those of blunt trauma patients with otherwise similar injury and demographic characteristics, and we implicate the chemokine IP-10/CXCL10 and the cytokine IL-10 in this phenomenon. Further, our data suggest that IP-10/CXCL10 and IL-10 may serve as biomarkers of adverse outcomes post-injury even in the absence of TSCI.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the University of Pittsburgh Institutional Review Board. Written informed consent was taken from each patient or the next of kin in line with our locally agreed protocols with Institutional Review Board regulations. Patients eligible for enrollment in the study were at least 18 years of age, admitted to the intensive care unit (ICU) within 24 h of injury, and per treating physician, were expected to live more than 24 h. Reasons for ineligibility were isolated head injury and penetrating trauma. Blunt trauma patients with and without TSCI from both trauma and neuro-trauma ICU's were enrolled in the study from June 2008 to November 2011.

Clinical Data Collection

This was a retrospective study involving a cohort of 569 trauma patients, 85 of whom had TSCI (68 males and 17 females, age 41 ± 1.9 , Injury Severity Score (ISS) 27.8 ± 1.7) and 484 of whom did not have TSCI (343 males and 141 females, age 48.4 ± 0.9 , ISS 19.8 ± 0.5). From this overall cohort, 42 survivors (21 TSCI and 21 non-SCI) were initially enrolled in our study and included in the analysis (Fig. 1). Exclusion criteria included patients with evidence of alcohol intoxication upon admission. Both groups were matched based on age, gender, and ISS, as shown in Table 1. Subsequently, we added 356 non-SCI patients (244 males and 112 females, age 50 ± 1 , ISS 17.5 ± 0.5) as well as 85 TSCI (68 males and 17 females, age 41 ± 1.9 , ISS 27.8 ± 1.7) in order to validate the findings of our initial analysis. Sequential clinical and laboratory parameters (see below) were collected during the subjects' 14 days post-injury. Clinical data, including ISS, ICU length of stay (LOS), hospital LOS, and days on ventilator were collected from hospital inpatient database. Laboratory results and other basic demographic data were recorded in the database via direct interface with electronic medical record. We used clinical criteria for suspected pneumonia (PNA) that included: new or progressive pulmonary infiltrates on radiograph after 48 h of hospital admission, and one or more of the following: fever, leukocytosis or leukopenia, and an increase in purulent endotracheal secretions. Clinically suspected pneumonia was diagnosed when a bacterial cell culture of $10,000$ colony forming units (cfu)/ml of bronchoalveolar lavage fluid was observed (14).

Analysis of cytokines, chemokines, and $\text{NO}_2^-/\text{NO}_3^-$

Blood samples were collected into citrated tubes via central venous or arterial catheters within 24 h of admission and daily for 14 days post-injury. The blood samples were centrifuged, and plasma aliquots were stored in cryoprecipitate tubes at -80°C for subsequent analysis of inflammatory mediators. The human inflammatory MILLIPLEX™ MAP Human Cytokine/Chemokine Panel-Premixed 26 Plex (Millipore Corporation, Billerica, MA) and Luminex™ 100 IS (Luminex, Austin, TX) was used to measure plasma levels of interleukin (IL)-1 β , IL-1 receptor antagonist (IL-1Ra), IL-2, soluble IL-2 receptor- α (sIL-2R α), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, interferon (IFN)- γ , IFN- γ inducible protein (IP)-10 (CXCL10), monokine induced by gamma interferon (MIG; CXCL9), macrophage inflammatory protein (MIP)-1 α (CCL3), MIP-1 β (CCL4), monocyte chemotactic protein (MCP)-1 (CCL2), granulocyte-macrophage colony stimulating factor (GM-CSF), Eotaxin, and tumor necrosis factor alpha (TNF- α). The Luminex™ system was

used in accordance to manufacturer's instructions. $\text{NO}_2^-/\text{NO}_3^-$ was measured using the nitrate reductase/Griess assay (Cayman Chemical Co., Ann Arbor, MI)

Statistical Analysis

All data were analyzed using SigmaPlot™ software (Systat Software, Inc., San Jose, CA). Group-time interaction of plasma inflammatory mediators' levels and injury was determined by Two-way analysis of variance (ANOVA). To quantify the differences between the statistically significant mediators, we calculated the area under the curve (AUC) using the mean values for each time point, and then calculating TSCI / non-SCI AUC fold changes. The correlation between different inflammatory mediators was determined by Spearman's correlation using the actual values of these inflammatory mediators. $P < 0.05$ was considered statistically significant for all analyses.

Dynamic Bayesian Network Inference

Dynamic Bayesian Network (DBN) inference was used to model the evolution of the probabilistic dependencies within a system over time. This analysis was carried out using MATLAB™ (The MathWorks, Inc., Natick, MA), using an algorithm adapted from Grzegorzczuk & Husmeier (15) and revised by our group(16). In this analysis, inflammatory mediators were represented at multiple time points within the same network structure. In this approach, time was modeled discretely as in a discrete Markov chain. Each mediator was given a time index subscript indicating the time slice to which it belonged. Additional temporal dependencies were represented in a DBN by edges between time slices. Each node in the network was associated with a conditional probability distribution of a variable that is conditioned upon its parents (upstream nodes). This particular network structure was used to assess the dominant inflammatory mediators and the probable interaction among various mediators, including possible feedback (15, 17).

Receiver Operator Characteristic (ROC) Curve

Receiver operator characteristic (ROC) curve analysis was carried out using MedCalc™ Software (MedCalc Software bvba, Ostend, Belgium) in order to evaluate the performance of the predictive model of patient morbidity based on circulating IP-10, with the goal of suggesting an optimal threshold and maximizing the sum of sensitivity and specificity (18). Accordingly, ROC analysis was carried out on the non-SCI group in order to determine the IP-10 value that could predict the worst clinical outcome suggested by prolonged total hospital LOS, ICU LOS, and days on the mechanical ventilation. Patients were divided randomly by the ROC into two groups to determine statistical significance (P value < 0.05 by Student's t -Test) in terms of total hospital LOS, ICU LOS, and days on the mechanical ventilator between the two random groups. Sensitivity and specificity of random cutoff values of circulating IP-10 were tested in order to classify non-SCI subjects into one of the two categories. The ROC analysis suggested that IP-10 levels ≥ 730 pg/ml (sensitivity 96.5% and specificity 98.5%, 95% CI) is the criterion for the optimal threshold separating worse outcomes from better outcomes (Fig. 7). To test this hypothesis, we segregated the remaining non-SCI and TSCI patients according to the ROC analysis cutoff.

RESULTS

Overview of demographics and outcomes in TSCI and non-SCI patients

The majority of patients in the current study were injured in motor vehicle accidents (57.1% and 80.9% in TSCI and non-SCI, respectively). Interestingly, falls were responsible for a greater proportion of the injuries in TSCI vs. non-SCI (38.1% and 14.3% in TSCI and non-SCI, respectively; $P = 0.002$) (Supplementary Materials, Table S1). Though there was a trend towards longer overall hospital stay, this was not significantly different between groups (38.5 ± 9.5 days vs. 26.8 ± 2.1 in TSCI and non-SCI, respectively). The TSCI group had a significantly greater requirement for mechanical ventilation than non-SCI group (33 ± 10 days vs. 10.3 ± 1.9 , respectively). Although ISS was matched in both groups, the Abbreviated Injury Scale (AIS) was significantly higher in non-SCI vs. TSCI, regarding abdominal, extremities, and external injuries (Fig. 2). With regard to the level of spinal cord injury in the TSCI group, 14 patients had injury at the level of the cervical spine while 7 patients had injury at the level of thoracic spine. Seventeen patients had complete spinal cord injury and 4 patients with incomplete one. One patient had documented anterior spinal cord syndrome, and two patients had complete spinal cord transection. The severity of the spinal cord injury was assessed according to the American Spinal Injury Association (ASIA) (19). Based on this classification, 17 TSCI patients had an ASIA score of A, one patient had ASIA score B, and 3 patients had an ASIA score of C. Eleven patients received non-steroidal anti-inflammatory drugs and systemic steroids in the TSCI group, vs. 15 patients in the non-SCI group ($P = 0.08$).

Different trajectories of systemic inflammation in TSCI and non-SCI patients

Blunt trauma induces a systemic inflammatory response (20), and this response is altered post-TSCI (21, 22). The differences in circulating inflammatory mediators between the two cohorts of patients are shown in Fig. 3. Interestingly, circulating IL-10 was significantly higher in TSCI when compared to non-SCI, whereas the concentrations of IL-1 β , sIL-2R α , IL-4, IL-5, IL-7, IL-13, IL-17, MIP-1 α and -1 β , GM-CSF, and IFN- γ were significantly lower in TSCI vs. non-SCI (Table 2).

Inflammatory Network inferred by DBN in TSCI and non-SCI

Based on these findings, we hypothesized that the differences in the dynamic, systemic inflammatory response between TSCI and non-SCI could be explained, at least in part, by differential network connectivity. Accordingly, DBN analysis suggested that MCP-1/CCL2 exhibited feedback on its own production and was the main driver for post-injury inflammatory response in non-TSCI, driving IL-1Ra, sIL-2R α , IP-10/CXCL10, MIG/CXCL9, MIP-1 β /CCL4, and Eotaxin. In contrast, IP-10/CXCL10 was inferred as the main driver of systemic inflammation in TSCI; in addition to increasing its own production, IP-10/CXCL10 also drove IL-10 (Fig. 4)

Divergent systemic inflammatory responses could explain increased risk for pneumonia in TSCI patients

We next examined the incidence of PNA in both groups, since this is a complication of blunt trauma (22, 23), and since TSCI patients are known to have higher incidence of pneumonia as compared to non-SCI patients (22, 24). The incidence of PNA in our study cohort was 81% in TSCI vs. 47.6% in non-SCI ($P = 0.002$) (Supplementary Materials, Table S3). In addition, the average onset of PNA was 9 ± 1.6 days post-injury in TSCI, whereas it was 5.4 ± 0.7 days post-injury in non-SCI patients ($P = 0.12$). Since inflammation was altered in TSCI vs. non-SCI patients, we hypothesized that the greatly altered systemic inflammatory response of TSCI patients as compared to that of non-SCI patients would predispose TSCI patients to an elevated risk of PNA. To begin to test this hypothesis, we examined the systemic inflammatory responses of TSCI patients with PNA (TSCI + PNA), and compared them to the systemic inflammatory responses of non-SCI patients with PNA (non-SCI + PNA). This comparison suggested that circulating IL-10 was significantly higher, whereas the concentrations of IL-1 β , sIL-2R α , IL-4, IL-5, IL-7, IL-13, IL-17, MIP-1 α and -1 β , GM-CSF, and IFN- γ were significantly lower in TSCI + PNA vs. non-SCI + PNA (Fig. 5). The dynamic networks inferred using DBN analysis showed that IP-10/CXCL10 was the main driver for TSCI + PNA (as in the TSCI cohort as a whole). Similarly, the core network of systemic inflammation in non-SCI + PNA was largely conserved as compared to the non-SCI cohort as a whole (Supplementary Materials, Fig. S1).

Systemic IP-10/CXCL10 is correlated positively with systemic IL-10 in TSCI but not in non-SCI patients

Dynamic Bayesian Network inference suggested that the systemic levels of IL-10 are driven by systemic IP-10/CXCL10 in TSCI. Therefore, we hypothesized that, on an individual basis and independently of the blood sampling time point, circulating levels of IL-10 would be correlated positively with circulating levels of IP-10. As shown in Fig. 6A, there was a positive correlation between IL-10 and IP-10/CXCL10 in TSCI patients ($r = 0.5$; $P < 0.001$). However, there was no apparent correlation between systemic IP-10/CXCL10 and IL-10 in non-SCI patients (Fig. 6B; $r = 0.04$; $P = 0.62$).

IP-10/CXCL10 segregates blunt trauma patients based on clinical outcomes

Since IP-10/CXCL10 was present in dynamic networks of both TSCI and non-SCI, we hypothesized that systemic IP-10/CXCL10 levels would segregate the clinical outcomes of blunt trauma patients. Accordingly, 233 non-SCI patients (156 males and 77 females, age 49.6 ± 1.3 , ISS 17.4 ± 0.6) and 45 TSCI patients (34 males and 11 females, age 42.9 ± 2.6 , ISS 26.4 ± 2.5) had circulating IP-10/CXCL10 values < 730 pg/ml. In contrast, 123 non-SCI (88 males and 35 females, age 50.9 ± 1.6 , ISS 17.8 ± 0.7) and 40 TSCI (34 males and 6 females, age 39.9 ± 2.9 , ISS 30.5 ± 2.4) patients had IP-10/CXCL10 levels ≥ 730 pg/ml across all time points.

The corresponding plasma IL-10 levels for both non-SCI groups are shown in Fig. 8A. Though there were no statistically significant differences in age and ISS between the non-SCI subgroups, patients with IP-10 levels ≥ 730 pg/ml had significantly prolonged hospital LOS (15 ± 0.9 vs. 9.6 ± 0.5 , $P < 0.001$), ICU LOS (7.7 ± 0.7 vs. 4.7 ± 0.4 , $P < 0.001$), and

days on mechanical ventilation (4 ± 0.6 vs. 1.8 ± 0.3 $P < 0.001$) vs. those with plasma IP-10 < 730 pg/ml (Fig. 8B). Similarly, TSCI patients with IP-10 > 730 pg/ml had significantly prolonged hospital LOS (27.9 ± 5.7 vs. 15 ± 2 , $P = 0.006$), ICU LOS (20.8 ± 5.2 vs. 10 ± 2.4 , $P = 0.03$), and days on mechanical ventilation (19.3 ± 5.8 vs. 9 ± 2.4 , $P = 0.029$) when compared to TSCI patients with plasma levels of IP-10 < 730 pg/ml (Fig. 8C).

DISCUSSION

In the present study, we compare for the first time detailed time courses of systemic inflammatory mediators in highly-matched, moderately-injured blunt trauma and TSCI patients. We find that the systemic inflammatory responses of these two cohorts of patients differ significantly and specifically: in thoraco-cervical TSCI patients, IL-10 is highly elevated, and most other inflammatory mediators measured are suppressed. Using a novel dynamic network inference algorithm, we suggest that the chemokine IP-10/CXCL10 drives systemic IL-10 in TSCI patients, while the chemokine MCP-1/CCL2 drives multiple mediators, including IL-6, in non-SCI patients. This latter observation is in agreement with a recent study from our group in which MCP-1 was implicated as a key inflammatory mediator produced by stressed hepatocytes, as a key regulator of IL-6 in these cells, and – at elevated levels – as a biomarker of morbidity in blunt trauma patients (25). In a similar fashion, we show that the chemokine IP-10/CXCL10 can segregate blunt trauma patients based on clinical outcomes, a finding that may suggest multiple chemokine-based processes as well as the presence of varying degrees of dysfunctional neural control of inflammation in these non-SCI patients.

A considerable number of studies have shown that the inflammatory response is modulated in part by a bidirectional communication between the central nervous system (CNS) and the immune system, through a hierarchy of neural and endocrine contacts (26, 27). Traumatic injury of the spinal cord induces local neuro-inflammation, which in turn triggers a cascade of secondary events, including breakdown of the endothelial barrier that surrounds the spinal cord (28), thereby propagating the inflammatory reaction systemically (29, 30).

Accordingly, we hypothesized that patients with TSCI would have distinct dynamic trajectory of systemic inflammation as compared to non-SCI trauma patients. The present study demonstrates quantitatively this significant difference, which manifests in significantly reduced circulating levels of multiple inflammatory mediators, supporting prior reports of immunodepression following SCI (31).

We suggest that this immunodepression is caused, at least in part, by elevated IL-10, which may serve other functions as well. Interleukin-10 exerts neuroprotective effects, either indirectly through its potent anti-inflammatory properties, or directly via effects on neurons that include reduction in apoptosis, reduction in caspase activation, and blocking NF- κ B activation (32). In support of this hypothesis, previous studies suggested that the lack of IL-10 in an animal model of SCI exaggerated secondary neuronal damage and worsened limb recovery (33).

Given the complex interplay among inflammatory mediators, we utilized DBN to infer causal interactions and suggest key control mechanisms. We leveraged DBN methodology,

whose clinical utility was demonstrated recently in the setting of pediatric acute liver failure (PALF). In that study, DBN of serum inflammatory mediators segregated spontaneous survivors from non-survivors when the raw mediator trajectories could not. Interestingly, this retrospective analysis showed that PALF patients selected for liver transplant had similar DBN to spontaneous survivors (16). In the present study, DBN inference led to the hypothesis that the elevated systemic IL-10 in TSCI is driven primarily by IP-10.

In support of our findings, individuals with plasma IP-10 levels ≥ 730 pg/ml had significantly prolonged total hospital, ICU length of stay, days on mechanical ventilator vs. those with plasma IP-10 < 730 pg/ml. IP-10 is a member of the CXC chemokine family, and is involved in the neural response to injury and inflammation (34, 35). An experimental study of TSCI in mice showed that IP-10 mRNA is unregulated early after TSCI (36). Multiple studies in experimental TSCI have suggested that IP-10 is induced in response to neuronal injury, and is involved in attracting T cells to the site of injury (37, 38). Thus, we hypothesize that locally-expressed IP-10 that is produced as part of a repair program may spill over systemically following spinal cord injury, driving systemic immunodepression. Thus, over-exuberant production of IP-10 may dampen inflammation excessively, resulting in predisposition to infection, impaired healing, or both.

Our *in silico* studies suggest that IP-10 drives the production or systemic release of IL-10, either directly or indirectly. Several studies have demonstrated the significant interaction of IP-10 and IL-10 in various experimental contexts (39, 40). These findings are in agreement with our observation that IP-10 induces the production of IL-10. In addition, there exists the possibility of IP-10/IL-10 positive feedback, since catecholamines can induce IL-10, which in turn can increase IP-10 production (41, 42).

In the current study, patients with TSCI had a higher incidence of various complications, chief among them being pneumonia (PNA). The later onset of PNA in TSCI could be associated with chronic exposure to mechanical ventilation (43). However, IL-10 rises shortly before the onset of PNA, suggesting a potential role of IL-10 in TSCI patients who develop nosocomial infection. TSCI patients with PNA exhibited a similarly blunted immune-inflammatory response even after the onset of infection, as did non-infected TSCI patients. Importantly, the DBN patterns of TSCI patients with or without PNA were also essentially identical; likewise, the DBN patterns of non-SCI patients without pneumonia were highly similar to those of non-SCI patients with PNA. Thus, it appears that, all else being equal, the presence or absence of TSCI (and not the development of PNA) is what determines the dynamic inflammation networks that evolve post-injury.

Although the impact of TSCI on the hospital length of stay was insignificant, LOS did trend towards being longer in TSCI patients vs. non-SCI. Furthermore, the higher incidence of deep venous thrombosis and pressure ulcers could reflect delayed functional recovery of patients with TSCI; future studies will investigate the impact of altered systemic inflammation post-TSCI on these other outcomes.

There are several limitations to the current study, including the number of inflammatory mediators analyzed; further studies examining a larger panel of inflammatory mediators are

warranted. In addition, our data were missing later time points of inflammatory mediators in non-SCI patients, specifically at 10, 11, and 12 days post-injury. We reasoned that by utilizing AUC analysis we could overcome this limitation and gain insights on the overall behavior of the inflammatory mediators across all time points between TSCI and non-TSCI. Furthermore, due to an IRB related limitation, we could not assess inflammatory mediators within the first 24 h post-injury as well as daily Marshall score of multi-organ dysfunction in TSCI group. Moreover, we do not have a qualitative assessment of the functional status of TSCI patients; rather, we used complications as a predictor of functional recovery. The study period was limited to 14 days, which represents the follow-up period in the acute care setting. Finally, we note that DBN inference can suggest possible interactions among inflammatory mediators, but this method does not explicitly show positive or negative influences.

In conclusion, the current study highlights a striking difference in the inflammatory trajectories associated with TSCI vs. injury of similar magnitude in the absence of TSCI. It is only through this comparison – and through the use of data-driven computational modeling – that we are able to appreciate the profound blunting of systemic inflammation post-TSCI. Ultimately, these insights may lead to better diagnosis and targeted therapeutic modulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Flow chart of recruitment and study participation. Both groups were matched according to age, gender, and injury severity score (ISS).

Figure 2.

Abbreviated Injury Scale (AIS) of traumatic spinal cord injury (TSCI) and non-SCI. Abdominal, extremities, and external injuries were assessed as part of the AIS, and were significantly higher in the non-SCI group as compared to the TSCI group. * $P < 0.05$ by Student's t-test.

Figure 3.

A. Time course of IL-10. **B.** Time course of IP-10. **C.** Time course of IL-17. **D.** Time course of IL-6. **E.** Time course of MCP-1. **F.** Time course of IL-5. **G.** Time course of IFN- γ . **H.** Time course of IL-1 β . **I.** Time course of MIP-1 β . Mean circulating levels of inflammatory mediators in both traumatic spinal cord injury (TSCI) and non-TSCI. Number of patients for each group: TSCI (n = 21), non-TSCI (n = 21). The indicated inflammatory mediators were assessed in serial plasma samples obtained at the indicated time points. Values are mean $\pm$ SEM. * $P < 0.05$ by Two-Way ANOVA.

Figure 4.

A. Dynamic Bayesian Network analysis (DBN) of traumatic spinal cord injury group suggests IP-10 as the main driver for inflammation that induces interleukin IL-10 production. **B.** DBN of non-SCI group suggests MCP-1 is the main driver of inflammation.

Figure 5.

Analysis of the subgroup with pneumonia. Number of patients for each group: traumatic spinal cord injury (TSCI) with pneumonia (n = 18), non-SCI with pneumonia (n = 10). **A.** Time course of IL-10. **B.** Time course of IP-10. **C.** Time course of IL-6. **D.** Time course of MCP-1. **E.** Time course of IFN-γ. **F.** Time course of IL-1β. **G.** Time course of IL-17. **H.** Time course of MIP-1β. Mean circulating levels of inflammatory mediators in both TSCI and non-SCI. The indicated inflammatory mediators were assessed in serial plasma samples obtained at the indicated time points. Values expressed as mean ± SEM *P < 0.05 by Two-Way ANOVA.

Figure 6.

A. correlation between IL-10 and IP-10 in traumatic spinal cord injury (TSCI) (n = 21). **B.** correlation between IL-10 and IP-10 in blunt trauma group without spinal cord injury (non-SCI) (n = 21).

Figure 7.

Receiver operator characteristic (ROC) curve for predicting clinical outcome based on IP-10/CXCL10. ROC analysis on circulating IP-10 levels suggested that IP-10 levels 730 pg/ml could separate non-SCI patients with better outcomes vs. non-SCI patients with worse outcomes (sensitivity 96.5% and specificity 98.5%; 95% CI).

Figure 8.

A. Plasma IL-10 is significantly ($P < 0.001$) lower in non-SCI patients with plasma IP-10 < 730 pg/ml ($n = 233$) vs. non-SCI patients with plasma IP-10 ≥ 730 pg/ml ($n = 123$). **B.** Non-SCI patients with plasma IP-10 levels ≥ 730 pg/ml had significantly prolonged hospital length of stay, ICU length of stay, and mechanical ventilator days vs. those with plasma IP-10 < 730 pg/ml. **C.** TSCI patients with plasma IP-10 levels ≥ 730 pg/ml had significantly prolonged hospital length of stay, ICU length of stay, and mechanical ventilator days vs. those with plasma IP-10 < 730 pg/ml. * $P < 0.05$ by Student's t-test.

Table 1

Demographic data, clinical characteristics and outcome in TSCI and non-SCI patients.

Variables	Traumatic spinal cord injury (TSCI)	Nontraumatic spinal cord injury (non-SCI)	<i>p</i>
Demographics			
Age, yr	37.2 ± 3.5	37± 2.2	0.91
Sex, male/female	16/5	16/5	1
Males age, yr	38.3 ± 3.9	37.9 ± 2.5	0.89
Females age, yr	33.6 ± 8	34 ± 5	0.9
Injury severity score (ISS)	32 ± 2.7	31 ± 2.8	0.69
Mechanism of injury			
Overall effect of mechanism of injury			0.007
Co-morbid conditions			
Patients with no co-morbidities, n (%)	12 (57.1)	14 (66.7)	0.4
Patients with co-morbidities, n (%)	9 (42.9)	7 (33.3)	
Outcome			
Mechanical ventilator, days	33 ± 10	10.3 ± 1.9	0.003
Hospital length of stay, days	38.5 ± 9.5	26.8 ± 2.1	0.69
Complications			
Patients with no complications, n (%)	1 (4.8)	4 (19)	0.09
Patients with complications, n (%)	20 (95.2)	17 (81)	

Table 2

Summarizes the folds of area under the curve (AUC) for the statistically different inflammatory mediators by Two-way ANOVA. IL-10, IL-5, IL-17, IL-4, IL-7, GM-CSF, IL-1 β , IL-13, MIP-1 β and 1 α , IFN- γ , soluble IL-2 receptor- α .

Inflammatory mediator	Two-way ANOVA p	Traumatic spinal cord injury (TSCI) Area Under Curve (AUC)	Non-traumatic spinal cord injury (non-SCI) Area Under Curve (AUC)	Fold of AUC (TSCI/ non-SCI)
IL-10	<0.001	1181.2	294.1	4
MIP-1 α	0.021	559.7	1192.1	0.46
sIL-2R α	<0.001	2571.7	7825.4	0.33
IFN- γ	0.021	325.5	1078.7	0.3
IL-1 β	0.014	152.8	588.3	0.26
IL-13	<0.001	140.8	579.2	0.24
MIP-1 β	<0.001	724.7	2988.6	0.24
IL-4	<0.001	155.9	778.9	0.2
IL-7	0.005	182.9	1015.1	0.18
GM-CSF	0.011	77.6	450.2	0.17
IL-5	0.014	92.3	579.6	0.16
IL-17	<0.001	161.9	1162.1	0.14