Research Article

Neonatal rats exhibit a predominantly anti-inflammatory response following spinal cord injury.

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Abstract

It has been reported that children may respond better than adults to a spinal cord injury (SCI) of similar severity. There are known biomechanical differences in the developing spinal cord that may contribute to this 'infant lesion effect', but the underlying mechanisms are unknown. Using immunohistochemistry, we have previously demonstrated a different injury progression and immune cell response after a mild thoracic contusion SCI in infant rats, as compared to adult rats. Here we investigated the acute inflammatory responses using flow cytometry and ELISA at 1h, 24h and 1week after SCI in neonatal (P7) and adult (9wks) rats and locomotor recovery was examined for 6 weeks post-injury. Adult rats exhibited a pronounced pro-inflammatory response characterised by neutrophils and M1-like macrophage infiltration, and Th1 cytokine secretion. Neonatal rats exhibited a decreased pro-inflammatory response characterised by a higher proportion of M2-like macrophages and reduced Th1 cytokine responses, as compared to adults. These results suggest that the initial inflammatory response to SCI is predominantly anti-inflammatory in very young animals.

Introduction

Pediatric spinal cord injury (SCI) accounts for only 1-13% of all reported SCI cases [1, 2] but spinal injury has a long and lasting impact on quality of life for these children. While it is acknowledged that children may be more vulnerable to severe injuries, if they survive an injury of comparable force, they appear to have a different, often better and faster recovery than their adult counterparts. A systematic review of published cases 1980-2011 reported that the "potential neurological recovery was higher in children compared to adults" [3]. Better functional recovery has also been reported following experimental spinal cord injury in young animals although the species, age range and methodology varied considerably between studies [4-8]

SCI is biphasal. There is an initial trauma resulting in primary injury, followed by an extended and evolving secondary injury phase with interactions between the central nervous system and the immune system [9-12]. A hallmark of the secondary injury phase is a heightened and sustained pro-inflammatory cascade, characterised by neutrophil and macrophage infiltration, microglial activation and astrogliosis. The functional states of macrophages are broadly categorised along a spectrum between the two basic phenotypes, M1 (pro-inflammatory, classically-activated) and M2 (anti-inflammatory, alternatively activated) [13]. M1-like macrophages secrete potentially neurotoxic effector molecules and pro-inflammatory cytokines, while the M2 phenotype is involved in dampening the inflammatory response and promoting wound healing and tissue remodelling [14]. The respective proportions of M1 and M2 macrophages determines the initiation and perpetuation of Th1/Th17 and Th2/Treg responses, respectively. Therefore, the temporal distribution and the magnitude of the M1-like and M2-like responses after SCI likely have significant roles in determining the efficacy and speed of injury resolution and the degree of functional recovery [15-17].

The different macrophage phenotypes are associated with different effector cytokines and chemokines. Pro-inflammatory Th1 modulators such as TNFα, IL-6, IL-1α/1β and IFN-γ are generally accepted to be detrimental to functional recovery when overexpressed or persistent [18]. These pro-inflammatory cytokines are not directly toxic to cells, but may be indirectly toxic through their interactions with cells when at high levels in the post-injury environment [19-21]. However, these cytokines also play a necessary role when they are kept in balance and a complete blockade of their activity may be detrimental to recovery. Anti-inflammatory, or Th2, cytokines such as TGF-β, IL-10, IL-4, and IL-13, are associated with wound healing, the injury resolution response, and alternatively activated macrophages [22, 14, 17, 23].In this study we investigated the acute (< 1 week post-injury) inflammatory responses in both neonatal and adult rats using flow cytometry and ELISA to determine if there were differences in the proportions of macrophage phenotypes, and pro- and anti-inflammatory cytokines following SCI. We also examined the locomotor function in animals after SCI to investigate patterns of motor function recovery.

Materials and Methods

Animals

46 Adult Sprague-Dawley rats (9 week old, female, ARC, Perth, Australia) and 76 infant (postnatal day 7, mixed sex,) were randomly allocated into groups for surgery (SCI or sham control groups) and were euthanized at one hour, 24 hours or one week post-surgery (n = 5 per adult group, n = 10 per infant group) for flow cytometry and cytokine analyses and at 6 weeks (n= 8 per group) for locomotor assessments. Infant samples were pooled with 2 spinal cords per sample for flow cytometry. During experimentation all animals were numbered and randomised as a blinding technique. All experimental procedures were performed with ethics approval from the University of Technology Sydney Animal Care and Ethics committee.

Surgery and euthanasia

Rats were anesthetised using 2% isoflurane with an oxygen flow rate of 1L/min. Local anaesthetic (0.2ml Bupivacaine, s.c) and iodine were applied to the shaved thoracic region. Prior to the surgery commencing, the rats were given analgesics (Buprenorphine hydrochloride -Temgesic 0.03mg/kg or (infants - 0.01mg/kg) body weight, s.c), antibiotics (Cephazolin sodium 33mg/kg body weight, s.c) and Hartman's replacement solution (Compound sodium lactate 15ml/kg body weight, s.c). An incision was made through the skin at the dorsal midline from the mid to low thoracic region and subsequent layers of tissue parted to expose the spinal column. This was followed by a bilateral laminectomy of the T10 vertebrae (adults) and mid-thoracic (infants) to expose the spinal cord with the dura left intact. The rats were then moved to the NYU/MASCIS weight-drop impactor, stabilised with clamps on the adjacent vertebrae and subjected to a weight-drop contusion injury. The height of the drop (6.5mm and 3mm) and diameter of the impactor head (2.5mm and 1mm) varied between groups to account for different sizes of spinal cords in order to produce an injury of comparable severity in animals as previously described using histology at 24 hrs [24]. Sham animals underwent all surgical procedures, except for the spinal impact. The surgical incision was sutured closed in layers (muscle, fascia, skin), and the animals were allowed to recover in a warm cage. Infant rats were washed in saline and exposed to home cage bedding material to remove any 'foreign' scents before being returned to their dams for post-operative care. All adult rats were given analgesics (Buprenorphine hydrochloride -Temgesic 0.03mg/kg body weight, s.c), antibiotics (Cephazolin sodium 33mg/kg body weight, s.c) and Hartman's replacement solution twice daily for at least 3 days and underwent manual bladder expression twice daily until normal voiding returned.

Flow Cytometry

Animals were euthanized at one hour, 24 hours and one week post-surgery using pentobarbital sodium (Lethabarb, 1ml/kg body weight,). The spinal cords were removed and

transferred to HBSS (Hank's balanced salt solution) buffer. A segment centred on the lesion site was manually sliced using sterile scissors, and then homogenised using the GentleMACS Dissociator (Miltenyi Biotec). Adult samples were processed singly while infant samples were pooled from this point onward, two animals to a sample, to ensure adequate volume of tissue for analyses of cell populations. The homogenate was then filtered and centrifuged (300xg for 10min) and the supernatant was collected for ELISA. The pellet was resuspended in 1ml of red blood cell lysis buffer for 5min at RT, centrifuged again, the supernatant removed, and the cells resuspended in HBSS. An Optiprep density gradient with solutions at four different concentrations was used to separate and isolate inflammatory cells as previously described [25, 26]. The top layer contained the cellular debris, which was discarded. Beneath this were layers of large neuronal cells, which were also discarded, leaving inflammatory cells and glia in the pellet. The inflammatory isolate was incubated with Sytox Blue, Mouse anti rat CD45-APC-Cy7 (Biosciences, 1:20), Mouse Anti-Rat CD68-FITC (Biorad 1:10), Mouse Anti-Rat CD86-PE (Biosciences, 1:20), Mouse Anti-Rat CD163-AF647 (Biorad 1:10) or Mouse Anti-Rat HIS48-FITC (Biosciences, 1:10), with appropriate isotypic controls (BD Biosciences). Compensation beads (CB) were used for the single colour controls (BD CompBeads #552843, BD Biosciences). Flow cytometry was performed on the samples using an LSRII Flow Cytometer (BD Biosciences) Gating was first conducted based on forward and side scatter properties of the cells, then SSC height and area to restrict to single cell events and then Sytox Blue for cell viability. Leukocytes were gated as $CD45^+$ and then specific populations of macrophages were discriminated using CD68, CD86 and CD163 and neutrophils using HIS-48. Activated macrophages were identified as $CD45^+/CD68^+$, the M1-like phenotype as $CD68^+/CD86^+$, and the M2-like phenotype as $CD68^+/CD163^+$ [27]. The relative numbers of activated macrophages, M1-like and M2-like macrophages was

determined. Neutrophils were identified as $CD45^{+/}HIS\text{-}48^{+}$ and were counted as a percentage of all viable cells.

Cytokine Analysis

Cytokine analysis was performed on the supernatant from the spinal cord homogenate using the Bio-Rad Pro Rat TH1/Th2 12-Plex panel for the Bio-Rad MagPix to quantify Th1 associated (M1) cytokines IFN-γ, IL-1α, IL-1β, IL-2, IL-6, IL-12 and TNF-α and Th2 associated (M2) cytokines IL-4, IL-5, IL-10, IL-13 and GM-CSF. The 96 well plate was prepared according to the manufacturers' specifications, using the 20x coupled beads and respective antibodies, with the supernatant diluted 1:2 with FSW and the standards prepared as 1:4 serial dilutions. The plate was analysed on the Bio-Rad MagPix using the standard settings recommended by the manufacturer.

Tissue analysis

The results for flow cytometry and ELISA will be presented as fold changes from the age appropriate sham animals. The size difference between P7 (14.8 +/- 1.6g) and adult rats (245.9 +/- 17.3g) presented issues in obtaining sufficient spinal cord tissue from the infant rats for direct comparison of absolute values. Pooled samples of multiple (~15) infant rats would have been required to obtain the same amount of tissue as a single adult rat spinal cord. Therefore, in order to reduce the number of experimental animals used we opted to examine data as fold changes only.

Hind-limb locomotor assessment

Adult rats were first acclimatised to an open field apparatus (90cm in diameter) where they were allowed to move around freely and naturally. Hind-limb locomotor function was assessed using the Basso Beattie and Bresnahan (BBB) Locomotor Rating Score [28] on the day prior to surgery to obtain a 'baseline' for each animal, and then on day 1, 3 and 7 post injury, followed by once a week for 6 weeks. Assessments were conducted using three minute

digital recordings by 2 independent assessors blinded to injury status. Infant rats were also assessed for locomotor function using the BBB scoring system, but it should be noted that this scoring system is designed and validated for adult rats. Sham (uninjured) rats had reached a score of 21 by 4 weeks of age indicating the development of mature hindlimb locomotor function. Therefore, a new 6 point scoring system for the infant rats was developed (Table 1) to compare the overground locomotor activity at the earlier timepoints. It was simple to implement and required minimal training using existing videos.

Data Analysis and Statistics

Changes in neutrophils were analysed using ordinary one-way ANOVA. Changes in leucocytes were analysed using two-way ANOVA (time and age) followed by Bonferroni's post hoc tests. For locomotor scoring two assessors independently analysed digital recordings and linear regression was used to confirm inter-observer variability (R^2 = 0.89-0.94) before averaging the results. Repeated measures ANOVA or mixed effects analysis was used to compare locomotor scores over time and by injury status with Bonferoni post hoc tests used to determine differences at each time point.

Results

Neutrophils

In the uninjured (sham) and at one hour post injury in the adult rat spinal cord neutrophils ($CD45^+/HIS48^+$) accounted for less than 0.5% of all viable cells while in the infant neutrophils accounted for less than 0.2% of all viable cells. In the injured adult spinal cords neutrophils increased to 4.3% +/- 2.8 (0.0124 v's sham) at 24 hours post injury while in the injured infant spinal cords neutrophils increased to 0.97% +/- 0.6 (p = 0.0186 v's sham) (Shown in Fig. 1).

Macrophage and Microglial Phenotypes

There were significant differences found in the inflammatory response for adults and infants (shown in Fig. 2). For $CD45^+$ leucocytes (shown in Fig. 2A) there were significant difference for both time ($F(2, 22) = 14.01$, $p = 0.0001$) and age ($F(1,22) = 5.640$, $p = 0.0267$). In the injured adult spinal cord, there was an increase in leukocytes from 0.06 fold at one hour to 6.5 fold at one week ($p < 0.0005$). In the infant rats there was a higher initial response (2.4 fold at one hour) that remained relatively steady (2.6 fold at one week, $p < 0.005$ v adults).

For M1-like ($CD68^+/CD86^+$) cells (shown in Fig. 2B) there were significant differences for both time ($F(2, 24) = 14.01$, $p = 0.0002$) and age ($F(1,24) = 8.765$, $p = 0.0068$). In the injured adult spinal cord, there was an increase in the fold change from shams from 0.3 fold at one hour to 8.2 fold at one week ($p < 0.0005$). In the injured infant spinal cord this increase was much smaller with a peak of only 2.7 fold at one week ($p<0.05$ v adults) (shown in Fig. 2B).

For the M2-like ($CD68^+/CD163^+$) cells (shown in Fig. 2C) the opposite pattern was observed. In the injured adult spinal cord, there was a small increase in the fold change from shams for M2-like cells (3 fold) at 24 hours that remained steady. In the injured infant spinal cord, there were increases in the fold change from shams for M2-like cells at 24 hours (5 fold) and at one week post injury (12 fold). This indicates a switch in the proportional response between the adults and infant rats whereby adults had a higher M1 response and infants had a higher M2 response at one week post injury compared to their own age appropriate controls.

Cytokines

The pro-inflammatory cytokine response for IL-1α, IL-1β, IL-6, IL-12 and IFN- γ and TNF-α, in adult and infant rat spinal cord, expressed as a fold change from the age appropriate sham controls are shown in Figures 3A and 3B. In the adult injured spinal cords, the level of all pro-inflammatory cytokines increased compared to sham controls with the largest increase observed for IL-1α levels (8 fold) at 1 week followed by TNF-α levels (6 fold) at 1 hour post injury. Elevated levels of some pro-inflammatory cytokines (notably IL-6 and IL-12)

persisted for up to one week post-injury. For the infant injured spinal cords there was a smaller pro-inflammatory cytokine response overall compared to adults with smaller increases for IL-1α (1.5 fold) at 1 week and TNF-α not measurable at 1 hour however TNF-α was elevated at later time points in the infant (~3 fold) at 24 hours and 1 week. The anti-inflammatory cytokine response for IL-4, IL-5, IL-10 and IL-13 are shown in Figures 3C and 3D. For the adult spinal cords, the highest increase in anti-inflammatory cytokines was for IL-13 (7 fold) at 1 week. In the infants the highest increase in anti-inflammatory cytokines were for IL-4 (2 fold) and IL-13 (4 fold) and these both peaked at one week post injury

Hind-limb Locomotor Function

All uninjured (sham) adult rats had a baseline BBB score of 21, and this was maintained over the six week period. The adult SCI rats had an average post-injury BBB score of 4.7 at day 1 post-injury, and this gradually increased to a final BBB score of 16.1 by week 6 post-injury (shown in Fig. 4A). There were significant differences in BBB scores between sham and SCI rats at each post injury time point (Bonferoni post hoc tests, $p < 0.0001$) These results are as expected and are consistent with what has previously been reported when the BBB scoring system is used to assess locomotor function following mild SCI in adult rats [29].

Uninjured (sham) infant rats developed full and measurable hindlimb locomotor function by 4 weeks of age as indicated by a BBB score of 21 (shown in Fig. 4B). The modified overground locomotor scores for infants (shown in Fig. 4C) showed a decrease in motor function (Bonferoni post hoc tests, $p < 0.01$) on day one post injury that normalised completely by two weeks.

Discussion/Conclusion

The findings from this study demonstrate that infant rats initiate and sustain an anti-inflammatory response to spinal cord injury as shown by a switch to a M2-like macrophage response and a lower Th1 cytokine response. In comparison, adult animals showed a

heightened pro-inflammatory response as indicated by an increased influx of neutrophils and an enhanced M1-like macrophage responses characterised by an increased Th1 cytokine response.

The acute pro-inflammatory response observed in the adult animals in the current study corroborates previous investigations of traumatic spinal cord injury in rats [10]. In the current study, SCI of adult rats was characterised by an early neutrophil infiltration, followed by a pro-inflammatory cytokine response, and then a macrophage activation/infiltration that has now been identified as predominantly M1-like in nature. It was apparent that in the adult rats $CD68^{+}/CD86^{+}$ M1-like macrophages gradually increased from one hour to one week following SCI. These classically activated M1 macrophages have been shown to persist for several weeks in other studies [17]. The smaller proportion of M2 macrophages seen in the adult spinal cords may be due to inhibition by the M1 macrophages, reduced phenotypic changes to resident macrophages or may represent a transient population of infiltrating blood-borne monocytes [30-32].

In contrast to this pattern of inflammation both the magnitude and the proportional response was quite different in the infant animals. The overall neutrophil response was lower in infants than for the adults at 24 hours post SCI. The difference in neutrophil numbers in infants may be attributable to the lower numbers of circulating neutrophils in younger animals, and also the lower numbers of granulocyte/monocyte colony forming units per gram of body weight [33-35]. In humans, the innate immune system at birth is biased against the production of pro-inflammatory cytokines, and this is important for protecting against unnecessary allogeneic immune reactions, and to facilitate tolerance to foreign, yet non-dangerous elements in the outside environment [36]. This was indeed the pattern observed in neonatal rats, with a lower local concentration of pro-inflammatory Th1 cytokines immediately after SCI. Following SCI a partial suppression or deletion of the neutrophil response and/or a reduction in associated

pro-inflammatory mediators can protect against some of the tissue damage that occurs during the secondary phase of injury [37-41]. Certainly in neonatal models of hypoxic-ischemic injury depletion of neutrophils has been shown to decrease brain swelling [42] and neutropenia in adults is associated with reductions in cerebral blood flow after stroke injury [43]. There are also indications that a reduction in neutrophil responses in very young animals are beneficial following myocardial infarction [44], lung disease such as bronchopulmonary dysplasia [45] and a M2-like macrophage phenotype contributes to normal postnatal development of alveolar tissue [46]. The reduction in neutrophils and Th1 cytokine levels is associated with an enhanced M2-like macrophage/microglia phenotype in the infant rat spinal cords that would be expected to be beneficial to injury resolution. P1 rats have also been shown to exhibit a less inflammatory and phagocytic phenotype in spinal cord derived microglia when compared to brain derived microglia in response to activation with lipopolysaccharide (LPS; a potent inducer of M1 macrophages) [47] and polarisation of spinal cord microglia to an M2 like phenotype has also been reported following a sciatic nerve injury in P10 rats with associated reductions in neuropathic pain [48, 49]. Recently several researchers have investigated regulation of microglial/macrophage polarization in the spinal cord to promote neuroprotection and functional recovery. A number of different factors have been administered including, for example, Melatonin, alpha-asarone, Atorvastatin and Naru-3, and all have reported to induce an increased M2-like macrophage/microglia phenotype and concomitant functional improvements [50-53]. Our previous immunohistochemical study showed that after injury the neonates had a higher proportion of resting microglia compared to adults rats [24] and the flow cytometry findings here indicate that the microglia/macrophages have a higher M2-like phenotype compared to adults rats following SCI.

Functional deficits in hindlimb locomotion were apparent in the adult rats following a mild T10 contusion injury and, while there was some spontaneous improvement, the injured rats

did not recover normal function over 6 weeks. In contrast there was little locomotor deficit observed in the infant rats following a mild SCI compared to the sham animals and there was no measurable difference in locomotor function once these rats had reached their maturity. The locomotor findings in this study suggest a very different pattern of injury and recovery exists in young animals compared to the adults. The main neurological pathways controlling locomotion are in place in P7 rats. Reticulospinal, vestibulospinal and rubrospinal tracts all reach the lumbar spinal cord before birth and mostly functional by P14 [54] and the corticospinal tracts have been reported to pass through the T10 level of the spinal cord by P7 [55]. The spinal cord of infant animals is significantly smaller and more flexible than the fully developed adult spinal cord [56] and accordingly the infant spinal cord moves and shifts under a weight-drop protocol used to induce SCI. In the current study, we modified the diameter of the impactor head and the height of the weight drop according to the rats' ages to produce injuries that were histologically similar to the adult at 24 hours post injury [24] suggesting that both adult and infant rats were subjected to a comparable mechanical insult to the spinal cord.

In the relatively few studies that directly compare infants to adults, the younger animals tend to have better neurological outcomes [4, 57-59, 7, 8, 60]. However, there are multiple factors that influence the development of locomotor function, and hence the development of locomotor deficits, in neonatal rats. These include the functional connectivity of descending neural pathways, the contribution of central pattern generators, maturity of the musculo-skeletal apparatus, postural adaption, myelination of axons and signalling cues. It has also been suggested that neonatal rats may be subjected to adaptive strategies that actually inhibit the development of complex locomotor function until about 2 weeks of age [61].

The normalised locomotor function that was observed in the maturing infant rats in the current study may represent re-organisation or possibly enhanced plasticity occurring after the

injury, and is complementary to the findings reported following a clip compression injury in mice [62, 63]. The predominantly anti-inflammatory response seen in the neonatal rats would provide a more permissive microenvironment for recovery compared to that seen following an adult spinal cord injury. Younger animals would not be exposed to the on-going secondary tissue damage associated with the strong pro-inflammatory response.

For future studies it would be useful to include a measurement of locomotor function without postural constraints such as air-stepping or a swim test [64, 62, 65] to better evaluate the locomotive deficit in the neonates immediately after SCI, as well as by using both retrograde and anterograde tracing to visualise the disruption (and re-organisation) to spinal tracts. Further research, including the use of Real-time PCR, more comprehensive flow cytometry with equal volumes of tissue to allow for absolute quantification of cell numbers rather than fold changes and detailed ELISA arrays is needed to elucidate the exact phenotypes of macrophage/microglial cells and to better quantify the cytokine/chemokine response. Adult female rats were used for this study but it would be important to compare the results with male rats given that sex-differences are known to exist in immune function.

To our knowledge the current study is one of only a few studies to compare the innate inflammatory response of infant rats or mice to adult animals following SCI. The results shown here suggest that infant rats have a larger anti-inflammatory response to spinal cord injury possibly driven by lower neutrophil numbers/infiltration.

Statements

All papers must contain the following statements after the main body of the text and before the reference list:

Acknowledgement (optional)

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Statement of Ethics

This research project was approved by the UTS Animal Care and Ethics Committee and follows the ARRIVE guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All authors had full access to the data in this study and take responsibility for the integrity of the data and accuracy of the data analysis. Study Concept and Design: T.S., B.O, C.G.; Acquisition of data: T.S., A.R., K.G. Analysis and interpretation of the data: T.S. and C.G. Drafting of the manuscript: T.S., C.G. and B.O. Obtained funding: T.S., C.G. and B.O.

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Figure Legends

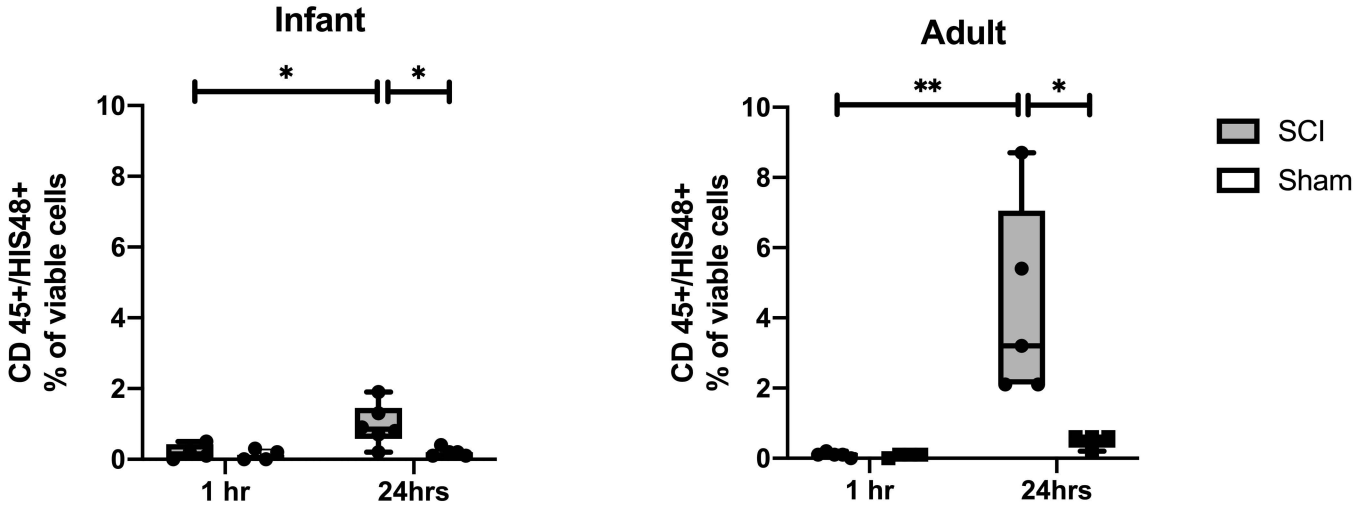
Fig. 1. Graphs showing the number of neutrophils as a percentage of viable cells following sham and spinal cord injury at 1 and 24hrs for A) infants and B) adult rats. Box graphs depict the median and the 25th and 75th quartiles with min/max values shown. * $p < 0.05$ Bonferroni's post-hoc test.

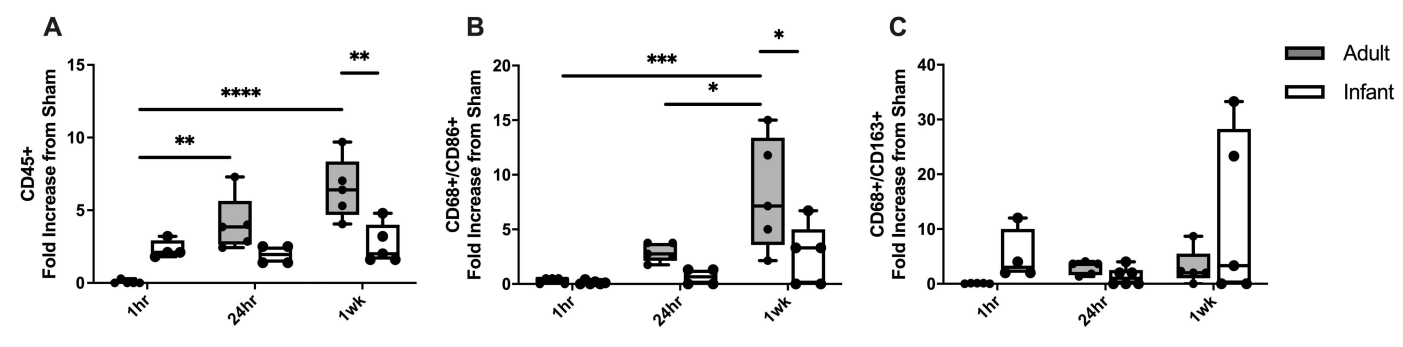
Fig. 2. Graphs showing the fold increase from sham levels of A) $CD45^+$ leukocytes, B) $CD68^+/CD86^+$ M1-like cells and C) $CD68^+/CD163^+$ M2-like cells as a percentage of the total viable cells in the adult and infant SCI groups. Box graphs depict the median and the 25th and 75th quartiles with min/max values shown. * ($p < 0.05$),** ($p < 0.001$) and *** ($p < 0.0001$), Bonferroni's post-hoc test.

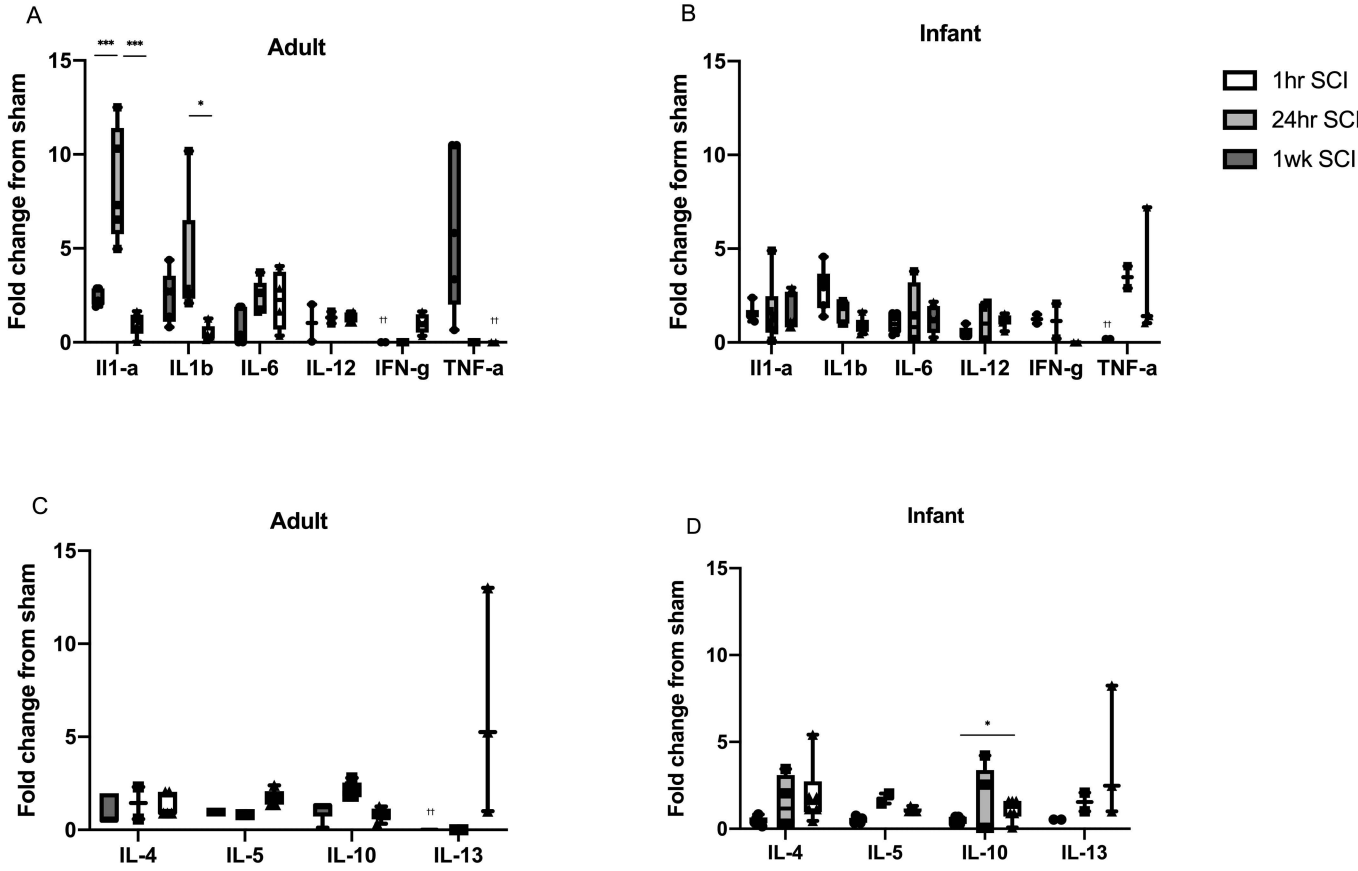
Fig. 3. Graphs showing the fold changes for pro-inflammatory cytokines IL-1α, IL-1β, IL-6, IL-12 with IFN- γ and TNF-α in A) adult spinal cords and B) infant spinal cords and for anti-inflammatory cytokines IL-4, IL-5, IL-10 IL-13, in C) adult spinal cords and D) infant spinal cords. Each graph shows the fold changes compared to the appropriate sham control at 24hrs, 1 day and 1 week post spinal cord injury. Box graphs depict the median and the 25th and 75th quartiles with min/max values shown.. Statistically significant differences at each time point are indicated * ($p < 0.05$), ** ($p < 0.001$) and *** ($p < 0.0001$), Bonferroni's post-hoc test. †† below detectable levels. SCI, spinal cord injury.

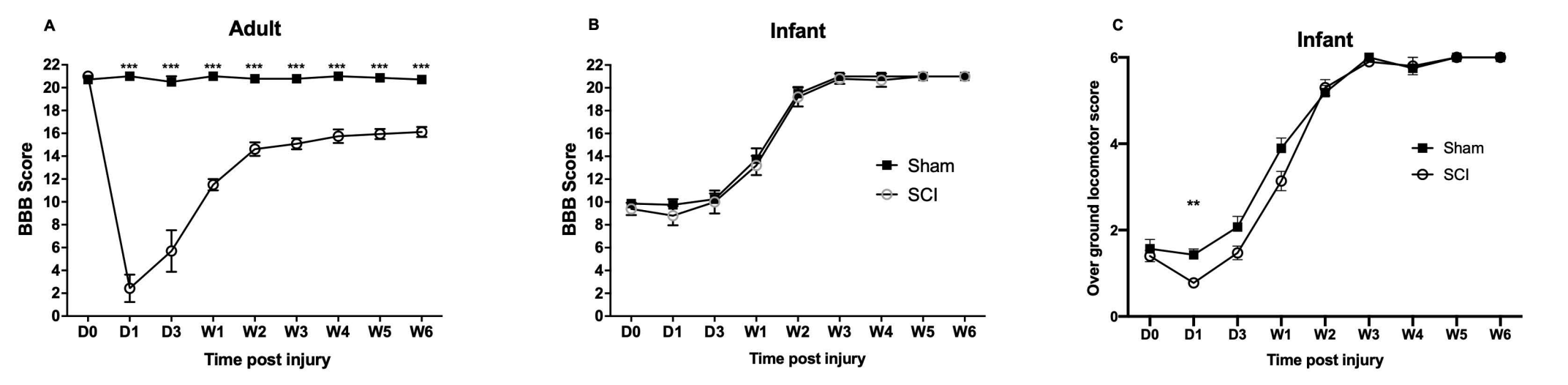
Fig. 4. BBB locomotor scores for A) adult and B) infant (P7) rats and C) overground locomotor scores for infant (P7) rats for six weeks following sham surgery or spinal cord injury. Error bars indicate SEM. Statistically significant differences at each time point are

indicated* ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.0001$), Bonferroni's post-hoc test. SCI, spinal cord injury.


Infant
Adult
CD 45+/HIS48+
% of viable cells
10
8
6
4
2
0
*
*
**
*
1 hr
24hrs
SCI
Sham


A
CD45+
Fold Increase from Sham
15
10
5
0
****
**
**
1hr
24hr
1wk
B
CD68+/CD86+
Fold Increase from Sham
20
15
10
5
0
***
*
*
1hr
24hr
1wk
C
CD68+/CD163+
Fold Increase from Sham
40
30
20
10
0
1hr
24hr
1wk
Adult
Infant


A
Adult
Fold change from sham
15
10
5
0
***
***
*
††
††
Il1-a
IL1b
IL-6
IL-12
IFN-g
TNF-a
B
Infant
Fold change form sham
15
10
5
0
††
Il1-a
IL1b
IL-6
IL-12
IFN-g
TNF-a
1hr SCI
24hr SCI
1wk SCI
C
Adult
Fold change from sham
15
10
5
0
††
IL-4
IL-5
IL-10
IL-13
D
Infant
Fold change from sham
15
10
5
0
*
IL-4
IL-5
IL-10
IL-13


A
Adult
BBB Score
Time post injury
D0
D1
D3
W1
W2
W3
W4
W5
W6
***
B
Infant
BBB Score
Time post injury
Sham
SCI
C
Infant
Over ground locomotor score
Time post injury
**
Sham
SCI