Acute and non-resolving inflammation associate with oxidative injury after human spinal cord injury

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Traumatic spinal cord injury is a devastating insult followed by progressive cord atrophy and neurodegeneration. Dysregulated or non-resolving inflammatory processes can disturb neuronal homeostasis and drive neurodegeneration. Here, we provide an in-depth characterization of innate and adaptive inflammatory responses as well as oxidative tissue injury in human traumatic spinal cord injury lesions compared to non-traumatic control cords. In the lesion core, microglia were rapidly lost while intermediate (co-expressing pro- as well as anti-inflammatory molecules) blood-borne macrophages dominated. In contrast, in the surrounding rim, TMEM119+ microglia numbers were maintained through local proliferation and demonstrated a predominantly pro-inflammatory phenotype. Lymphocyte numbers were low and mainly consisted of CD8+ T cells. Only in a subpopulation of patients, CD138+/IgG+ plasma cells were detected, which could serve as candidate cellular sources for a developing humoral immunity. Oxidative neuronal cell body and axonal injury was visualized by intracellular accumulation of amyloid precursor protein (APP) and oxidized phospholipids (e06) and occurred early within the lesion core and declined over time. In contrast, within the surrounding rim, pronounced APP+/e06+ axon-dendritic injury of neurons was detected, which remained significantly elevated up to months/years, thus providing mechanistic evidence for ongoing neuronal damage long after initial trauma. Dynamic and sustained neurotoxicity after human spinal cord injury might be a substantial contributor to (i) an impaired response to rehabilitation; (ii) overall failure of recovery; or (iii) late loss of recovered function (neuro-worsening/degeneration).
Introduction

Traumatic spinal cord injury (SCI) is a devastating condition with a substantial economic burden impacting on the physical and psychological well-being of patients (Ahuja et al., 2017; James et al., 2019). The initial mechanical injury is not only followed by classical secondary injury (Ort and Gensel, 2018) but also by long-standing progressive neurodegeneration (Freund et al., 2013; Ziegler et al., 2018) as reported for a subpopulation of traumatic SCI patients presenting with continued worsening of neurological functions even >6 months post-injury (Marino et al., 1999; Kirshblum et al., 2004). Evidence from experimental studies suggests that limiting pro-inflammatory bystander damage can improve SCI outcome (Gris et al., 2004; Kerr et al., 2008; David et al., 2012; Ramer et al., 2014).

Within the first hours to days after the insult in both humans and experimental animals, polymorphonuclear neutrophils (PMNs) accumulate at the lesion site and peak within the first 24 h (Taoka et al., 1997; Fleming et al., 2006; Beck et al., 2010). Microglia activation and monocyte/macrophage infiltration follow during the first days and their numbers remain elevated up to months post-SCI (Fleming et al., 2006; Pruss et al., 2011). Similar patterns have been described for T cells in human SCI (Fleming et al., 2006), although significant species differences between mice, rats and humans have been described in regard to lymphocyte composition as well as spatiotemporal patterns (Sroga et al., 2003). Although a potential peripheral autoimmune response has been discussed in human SCI (Zajarias-Fainsod et al., 2012), lesion-associated B cells have not been observed in the only available neuropathological assessment of human SCI thus far (Fleming et al., 2006). This is in contrast to experimental SCI models, for which B cell accumulation and plasma cell conversion with concomitant IgG autoantibody production has already been shown (Ankeny et al., 2006, 2009).

Microglia and CNS-infiltrating blood-derived macrophages arise from two ontogenetically distinct populations and can adopt a gradient from a pro-inflammatory phenotype associated with cytotoxicity and a potential beneficial anti-inflammatory phenotype (Kigerl et al., 2009; Ginhoux et al., 2010; Kroner et al., 2014; Ransohoff, 2016). In experimental SCI models, an M1 pro-inflammatory polarization of microglia/macrophages prevails (Kigerl et al., 2009). So far, polarization patterns in human SCI cases have not been studied. The distinction between purely M1 and M2 phenotypes is anyhow oversimplified and not advisable for in vivo studies (Ransohoff, 2016; Zrzavy et al., 2017, 2018). Instead, microglia/macrophages should be characterized according to their functional properties, e.g. by means of the homeostatic marker P2RY12, which is lost in a pathological CNS environment (Fleming et al., 2006; Beck et al., 2010; Krasemann et al., 2017). Under neuroinflammatory conditions, microglia and macrophages dominantly express molecules involved in the production of reactive oxygen species. Oxidative neuronal injury is triggered by reactive oxygen species resulting in lipid peroxidation and intracellular accumulation of oxidized phospholipids. Oxidative neuronal injury is well documented for human CNS injury and associated with activated microglia and macrophages (Haider et al., 2011; Jia et al., 2012).

Most of our understanding of the inflammatory response after SCI derives from preclinical experiments, which do not always reflect the human CNS pathophysiology (Dirnagl, 2014; Schuh et al., 2014). Detailed information on human traumatic SCI, however, is sparse and molecular mechanisms contributing to delayed neurodegeneration are likewise unknown, which poses a fundamental void in our understanding of the pathophysiology after human SCI. To fill in missing links, we performed a systematic analysis of (i) the recruitment of leucocytes into human SCI lesions; and (ii) the spatiotemporal heterogeneity of microglia and macrophages by applying a validated panel of functional markers. Moreover, we characterized the kinetics of oxidative tissue injury by using oxidized lipids as validated and established marker for neuronal cell body and axonal injury (Haider et al., 2011; Friese et al., 2014).

Material and methods

Sample characterization

Our study was performed using an archival collection of spinal cord autopsy tissues derived from 22 patients with traumatic SCI and five non-traumatic control patients; all collected during the past decades in the archive of the Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna. All available medical records, laboratory charts and autopsy charts were screened. Non-traumatic control cases were comprehensively examined for any pathological alterations in the white and grey matter, particularly inflammatory infiltrates and signs of tissue injury, for which tissue sections were routinely stained with haematoxylin and eosin, Luxol fast blue and Bielschowsky silver impregnation. Patient demographics, time of death post-SCI and lesion level are summarized in Table 1. The study was approved by the ethics committee of the Medical University of Vienna (EK. Nr.: 1454/2018 and 1636/2019).

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections with a biotin/avidin detection system as described in detail before (Bauer and Lassmann, 2016) using primary antibodies and respective antigen retrieval methods as listed in Supplementary Table 1. For lymphocyte characterization, we used antibodies targeting CD3 (T cells), CD4 (MHC class II-restricted T cells),
CD8 (MHC class I-restricted T cells), CD20 (B cells), CD79a (B cells and plasma cells) and CD138 (plasma cells). Lymphocytes were distinguished from macrophages, which can express CD4 or CD8 as well, by morphological criteria and a lack of CD68 expression. C9neo staining was used to identify complement-mediated tissue injury (Bien et al., 2012). Granulocytes were detected by their characteristic appearance (segmented cell nuclei) in tissue sections stained with haematoxylin and eosin. For the characterization of myeloid cells, Iba-1 was used as a general microglia/macrophage marker; TMEM119 and P2RY12 were used as microglia-specific markers. TMEM119 is a transmembrane protein with unknown function expressed on microglia, but not on recruited myeloid cells in humans and rodents (Butovsky et al., 2014; Bennett et al., 2016). During microglia activation, TMEM119 expression is downregulated; however, a residual level is preserved on the surface (Satoh et al., 2016). Resting microglia were identified using P2RY12, which is rapidly lost upon activation in experimental models of CNS inflammation or neurodegeneration (Krasemann et al., 2017; Zrzavy et al., 2017). CD68 was used to detect phagocytic activity; HLA-DR and CD86 were used to identify cells involved in antigen presentation, processing and co-stimulation. Furthermore, p22phox (NADPH oxidase) and inducible nitric oxide synthase (iNOS) were used as markers for oxidative activation and signs of oxidative tissue injury. Anti-e06 antibodies were applied to detect oxidative injury (intracellular loading with oxidized phospholipids) in neurons (Haider et al., 2016). As prototypical anti-inflammatory markers, expression of the haemoglobin-haptoglobin receptor CD163 and the mannose receptor CD206 were investigated (Zrzavy et al., 2018). Moreover, staining for the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) as well as the anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor beta (TGF-β) were performed (Machado-Santos et al., 2018; Trösch et al., 2019). All markers have already been extensively characterized regarding their reliability and expression patterns in human autopsy material of lymphatic tissue (Zrzavy et al., 2017). For control, immunohistochemistry was performed by omitting of primary antibodies and by using normal rat and goat serum or isotype-matched monoclonal antibodies.

### Table 1 Clinical demographics

| Case   | Level | Cause                        | Days post-injury | Cause of death                  | Age  | Sex | Type of injury     |
|--------|-------|------------------------------|------------------|---------------------------------|------|-----|-------------------|
| SCI-1  | Th    | Gun shot                     | <1               | Cardiovascular failure          | 33   | M   | Massive compression |
| SCI-2  | C     | Fall                         | 1                | Cardiovascular failure          | 79   | M   | Contusion/cyst     |
| SCI-3  | C     | Fall                         | 2                | Respiratory insufficiency       | 30   | M   | Contusion/cyst     |
| SCI-4  | C     | Fall                         | 2                | Pulmonary embolism              | 79   | F   | Massive compression |
| SCI-5  | C     | Fall                         | 4                | Cardiovascular failure          | 53   | M   | Contusion/cyst     |
| SCI-6  | C     | Fall                         | 4                | Cardiovascular failure          | 75   | F   | Contusion/cyst     |
| SCI-7  | C     | Fall                         | 5                | Pulmonary embolism              | 50   | M   | Contusion/cyst     |
| SCI-8  | TH    | Massive prolapsed disc       | 13               | Pulmonary embolism              | 35   | F   | Massive compression |
| SCI-9  | C     | Fall                         | 14               | Unknown                         | 45   | M   | Contusion/cyst     |
| SCI-10 | C     | Fall                         | 15               | Cardiovascular failure          | 37   | M   | Contusion/cyst     |
| SCI-11 | C     | Fall                         | 19               | Unknown                         | 15   | M   | Contusion/cyst     |
| SCI-12 | C     | Fall                         | 18               | Cardiovascular failure          | 71   | M   | Contusion/cyst     |
| SCI-13 | L     | Car accident                 | 18               | Renal failure                   | 19   | M   | Contusion/cyst     |
| SCI-14 | C     | Fall                         | 25               | Respiratory insufficiency       | 39   | M   | Massive compression |
| SCI-15 | C     | Fall                         | 36               | Respiratory insufficiency       | 22   | M   | Contusion/cyst     |
| SCI-16 | C     | Fall                         | 48               | Pulmonary oedema                | 80   | M   | Contusion/cyst     |
| SCI-17 | C     | Fall                         | 89               | Hepatorenal failure             | 65   | M   | Contusion/cyst     |
| SCI-18 | C     | Fall                         | 91               | Pulmonary embolism              | 61   | M   | Contusion/cyst     |
| SCI-19 | TH    | Car accident                 | 210              | Pulmonary oedema                | 34   | F   | Contusion/cyst     |
| SCI-20 | C     | Car accident                 | 352              | Cardiovascular failure          | 24   | F   | Contusion/cyst     |
| SCI-21 | C     | Massive prolapsed disc       | 365              | Cardiovascular failure          | 56   | M   | Contusion/cyst     |
| SCI-22 | C     | Fall                         | 413              | Cardiovascular failure          | 58   | M   | Contusion/cyst     |
| CO-1   | C     |                              |                  | Cardiovascular failure          | 53   | F   |                   |
| CO-2   | C     |                              |                  | Renal failure                   | 40   | M   |                   |
| CO-3   | C     |                              |                  | Hepatic failure                 | 73   | M   |                   |
| CO-4   | C     |                              |                  | Cardiovascular failure          | 70   | F   |                   |
| CO-5   | C     |                              |                  | Hepatic failure                 | 32   | M   |                   |

C = cervical; F = female; L = lumbar; M = male; Th = thoracic.
same species (TMEM119, p22phox, APP, e06), heat-induced epitope retrieval was performed in between the separate antibody incubations and subsequent immunohistochemical detection reactions according to Bauer and Lassmann (2016).

**Immunofluorescence**

For fluorescence double or triple stainings, with two antibodies from the same species, extensive heat-induced epitope retrieval in between the subsequent immunohistochemical reactions was performed. After deparaffinization and blocking of non-specific binding, the first round of antigen retrieval (Supplementary Table 1) was done and tissue sections were incubated with primary antibodies against Iba-1, P2RY12 or IL-18. This was followed by incubation with biotinylated anti-rabbit antibody and avidin peroxidase as well as catalysed signal amplification with biotinylated tyramine (Bauer and Lassmann, 2016). Then, a second round of antigen retrieval (30 min; EDTA buffer pH 9) was done, followed by incubation of tissue sections with Cy2- or Cy3-conjugated streptavidin. Thereafter, the second round of primary antibodies (anti-TMEM119, anti-GFAP or combinations from different species) were applied and visualized by subsequent incubation with Cy3 or Cy5-conjugated anti-rabbit, antigoat or anti-mouse secondary antibodies (Trösch et al., 2019; Zrzavy et al., 2019). Finally, sections were mounted with Prolong™ Gold antifade mountant (Thermo Fisher Scientific, P36930) and visualized with a confocal laser microscope (Leica SP5, Austria).

**Turnbull blue staining**

3,3’-Diaminobenzidine tetrahydrochloride hydrate (DAB)-enhanced Turnbull’s blue (TBB) staining for the detection of non-haem iron was done as described (Hametner et al., 2013).

**Quantitative assessment**

Regions of interest included the lesion core (‘core’) as well as the directly surrounding tissue margin (‘rim’) (Fig. 1). The core region corresponds to the definition of ‘Zone 1’ and the rim region to the definition of ‘Zone 2’ as described previously (Fleming et al., 2006). The spinal cords were classified into the following stages (time windows) after SCI displaying consistent histopathology: (stage I) 1–3 days post-SCI ($n = 4$); (stage II) 4–21 days post-SCI ($n = 9$); (stage III) 21–90 days post-SCI ($n = 4$); and (stage IV) 90 days to 1.5 years post-SCI ($n = 5$) compared to controls ($n = 5$). For the quantitative evaluation of microglia/macrophages, sections were overlayed by a morphometric grid (0.2256 mm²) placed within the ocular lens and two to three fields per region of interest were selected. Cells expressing the respective marker were manually counted and the values are expressed as cell counts per square millimetre. For quantification of lymphocytes, a morphometric grid within the ocular lens was used as well and inflammatory cell numbers were manually counted in 5–20 fields of 0.2256 mm² per region of interest spanning the entire lesion. The number of T cells and B cells were counted separately for perivascular and parenchymal areas and were pooled afterwards.

Digital optical densitometry was performed for quantification of oxidized phospholipids (e06 immunoreactivity) according to a previously published protocol (Hametner et al., 2013). This marker was quantified by calculating the positive DAB signal area fraction using ImageJ. One to two images per region of interest were taken with standardized acquisition settings. For digital removal of haematoxylin counterstaining, a colour deconvolution plugin (freeware kindly provided by A. C. Ruifrok, NIH) was run. Further RGB images were converted into 8-bit greyscale images and inverted. A threshold was set, which was kept constant between analyses; subsequently, the area fraction of the resulting images was calculated.

**Statistical analysis**

Statistical analysis was performed with IBM SPSS 20.0.0 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism® v6.01. Because of uneven distribution of our data, statistical analysis was performed with non-parametric tests. Descriptive analysis included median value and range. Differences between two groups were assessed with Wilcoxon Mann-Whitney U-test. In case of multiple testing, significant values were corrected with the Bonferroni-Holm procedure. The reported $P$-values are results of two-sided tests. A $P$-value $\leq 0.05$ was considered statistically significant.

**Data availability**

Data can be made available from the corresponding author upon reasonable request and after approval from the ethics review board at the Medical University of Vienna.

**Results**

For the analysis of inflammatory and degenerative cascades following traumatic SCI, we examined an archival tissue collection of 22 traumatic SCI and five non-traumatic control cases (Table 1). The mean age of the SCI cohort was 48.1 years (range 13–80 years), which was slightly younger than the control cohort (53.4 years, range 32–73 years). The gender distribution ratio was 16:6 (male: female) in the spinal injury cohort, which matches epidemiological data (Ahuja et al., 2017), and 3:2 (male: female) in the control group. All analysed tissue blocks contained the actual impact zone of SCI. Since (i) it is impossible to reliably distinguish between grey and white matter in the case of severe SCI; and (ii) we did not see significant differences in microglia numbers between normal spinal cord grey and white matter (data not shown), we did not differentiate between the two areas in further analyses. Putative control cases were solely admitted.
Figure 1 Spatiotemporal characterization of neuropathological features in human SCI lesions. The sequence of neuropathological alterations was grouped into four stages (I–IV). An overview of the progressive lesion evolution is illustrated by haematoxylin and eosin (H&E)-stained spinal cord cross-sections (middle row) accompanied by higher-resolution details of the corresponding lesion core (left) and surrounding rim (right). Anterior spinal cord is facing downwards, while dorsal cord is directed upwards. Stage I (1–3 days post-SCI): (A) Distortion and disruption of parenchyma and inherent vessels with ensuing haemorrhage and leukoapthaposis (extravasation, arrows) in the lesion core. Hyperpolarized red blood cell are detected outside of the neurovascular unit in a radial manner in close vicinity to the damaged vessels (B). Abundant swollen hypertrophic β-APP + axonal spheroids (black arrow) indicate axonal injury. These ballooned retraction bulbs indicate severe cytoskeletal disruption and are intermingled with β-APP + axons displaying features (dotted segregated β-APP + alignments; green arrow) in line (continued)
to the control cohort if pathological changes, particularly inflammatory infiltrates and signs of tissue injury, which would have been detectable in haematoxylin and eosin staining, Luxol fast blue myelin staining and Bielschowsky silver impregnations, could be fully excluded.

Different spatiotemporal dynamics of neuropathology evolve after human spinal cord injury

For a rough categorization, the sequence of neuropathological alterations after traumatic SCI, which include inflammation, neurodegeneration and tissue damage, can be grouped into four stages (I–IV): SCI of stage I (1–3 days post-SCI) was characterized by distortion and disruption of parenchyma integrity, haemorrhage, and leukodiapedesis (extravasation) in the lesion core (Fig. 1A and B) with abundant β-APP+ axonal spheroids indicating axonal injury (axonal blebbing) (Fig. 1C). In the lesion-surrounding white matter, commencing myelin damage (demyelination) with early 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) loss was evident (Fig. 1D); expression of proteolipid protein (PLP) was still preserved (Fig. 1E). Injured spinal cords of stage II (4–21 days post-SCI) demonstrated tissue necrosis within the lesion core with typical spongiotic tissue changes (Fig. 1F) concomitant with ongoing debris removal evidenced by large numbers of CD68+ macrophages containing large lysosomal vacuoles (Fig. 1G). Within the lesion rim, abundant β-APP+ axonal spheroids with various elongations (‘axonal varicosity’) (Fig. 1H) and scattered CD68+ macrophages (Fig. 1I) were present. Stage III (21–90 days post-SCI) was characterized by progressive spongiotic changes with numerous CD68+ macrophages in the lesion core (Fig. 1J and K). However, in comparison with stage II, the number of CD68+ macrophages had already subsided. In the lesion rim, remaining axonal spheroids were surrounded by reactive gliosis composed of hypertrophic activated GFAP+ astocytes (Fig. 1L and M), which extended vascular end-feet with pronounced bouton-type appearance in order to support vascular integrity (stabilization of the neurovascular unit) (Fig. 1M). Finally, stage IV (90 days to 1.5 years post-SCI) demonstrated cystic cavitation (syrinx) in the lesion centre. This scar was surrounded by a hypercellular rim (Fig. 1N) containing non-resolving, activated CD68+ macrophages. (Fig. 1O). They also formed clusters in Virchow-Robin-alike spaces pointing to a chronic immunological activation (Fig. 1O). The lesion rim showed massive reactive GFAP+ gliosis and a remainder of evenly distributed CD68+ macrophages was still present (Fig. 1P and Q). Spinal cord tissue from controls did not contain any pathological changes (not shown). We further extended the basic spatiotemporal characterization of human traumatic SCI lesions with more detailed analyses of cellular compositions and activation patterns as well as of oxidative tissue injury.

Polymorphonuclear neutrophils

PMNs were absent in non-traumatic control spinal cords (Figs 2A and 3A). After traumatic SCI, they were readily present, peaked within the first 3 days after injury in the lesion core (P = 0.032) (Figs 1A, B and 2A) and were also detectable in some cases within the lesion rim (Fig. 3A). The number of PMNs declined during the first 3 weeks post-SCI and they were not observed at any later time points.
Figure 2 Quantitative analysis of cellular markers in the spinal cord lesion core. Quantitation of PMNs and various microglia/macrophage marker expression in the core of SCI lesions at different lesion stages (I–IV) as well as in non-traumatic control tissue. Data represent actual numbers of cells/mm². (A) PMNs counted in haematoxylin and eosin staining, (B) pan-microglia/macrophage marker Iba-1, (C) the microglia-specific marker TMEM119, (D) the 'homeostatic' microglia marker P2RY12; and the microglia activation markers (E) p22phox, (F) CD68, (G) HLA-DR, (H) CD86, (I) iNOS, (J) CD163, (K) CD206 and DAB-enhanced TBB staining for the detection of non-haem iron (L). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
Figure 3 Quantitative analysis of cellular markers in the spinal cord lesion rim. Quantitation of PMNs and various microglia/macrophage marker expression in the core of SCI lesions at different lesion stages (I–IV) as well as in non-traumatic control tissue. Data represent actual numbers of cells/mm². (A) PMNs counted in haematoxylin and eosin staining. (B) pan-microglia/macrophage marker Iba-1. (C) the microglia-specific marker TMEM119. (D) the ‘homeostatic’ microglia marker P2RY12; and the microglia activation markers (E) p22phox, (F) CD68, (G) HLA-DR, (H) CD86, (I) iNOS, (J) CD163, (K) CD206 and DAB-enhanced Turnbull Blue (TBB) staining for the detection of non-haem iron (L). *p < 0.05; **p < 0.01; ***p < 0.001.
analysed. PMNs were located perivascularly; in some cases, they were detected in the parenchyma as well.

**Microglia and blood-derived macrophages in the lesion core and surrounding lesion rim**

In non-traumatic control patients, Iba-1+ cells exhibited a ramified microglia phenotype and co-expressed the microglia marker TMEM119 (Fig. 4A). Most of these TMEM119+ cells were positive for the homeostatic marker P2RY12 as well (Fig. 4B). In the necrotic core of stage I lesions, we detected a significant loss of Iba-1+ cells (P = 0.032), while their numbers remained constant in the surrounding rim (Figs 2B and 3B). The vast majority of Iba-1+ cells in both lesion core and rim were TMEM119+ (average 81% and 89%, respectively) (Figs 2C and 3C). At stage II, there was a massive increase of Iba-1+ cells within the lesion core as well as rim (P = 0.019 and P = 0.002, respectively) (Figs 2B and 3B). However, while 80% of Iba-1+ cells within the lesion rim were also TMEM119+, this number dropped to 10% within the lesion core. Morphologically, cells in the lesion core were characterized by an amoeboid shape i.e. oval to round cell bodies without processes (Fig. 4D and H). This indicates a substantial recruitment of peripheral TMEM119+ macrophages into the lesion core, while the increased numbers of Iba-1+ TMEM119+ cells within the lesion rim was mostly due to local microglia proliferation as shown by proliferating cell nuclear antigen (PCNA) expression (Fig. 4J) in 17% of TMEM119+ cells. At stage III, numbers of Iba-1+ cells were still significantly increased in both the lesion core and rim (P = 0.32 and P = 0.32, respectively) (Figs 2B and 3B). From stage II to stage III, there was a further decline of TMEM119+ co-expressing Iba-1+ cells to 5% in the lesion core; several months post-SCI (stage IV), we could not detect any more TMEM119+ cells in this area (Fig. 2C). In the rim, we observed that from stage II to IV, approximately half of Iba-1+ cells stably co-expressed TMEM119 (stage II, 80%; stage III, 49%; stage IV, 47%). Results show that microglia (Iba-1+ TMEM119+ cells) constituted a substantial proportion of myeloid cells in the edge during lesion maturation, while recruited blood-borne monocytes/macrophages (Iba-1+ TMEM119− cells) dominated within the lesion core.

**Differential patterns of microglia and macrophage activation**

In the lesion core, a rapid and long-lasting loss of P2RY12 (homeostatic marker) expression on myeloid cells was detected (stage I, P = 0.032; stage II, P = 0.002; stage III, P = 0.032; stage IV, P = 0.016) (Figs 2D, 4E and F). A similar pattern was observed in the lesion core, however low numbers of P2RY12+ cells reappeared in some cases at later time points (stage IV) (Fig. 3D).

Within the core of tissue injury, a significant pro-inflammatory activation of myeloid cells, the vast majority of which were TMEM119+, was detected by an upregulation of p22phox, CD86, MHC class II and CD68 expression (Figs 2E–H and 4). Numbers of iNOS+ myeloid cells also increased during stages II and III, albeit to much lower extents (Fig. 2I). Concomitant with pro-inflammatory markers, we also observed an increased expression of the anti-inflammatory markers CD163 and CD206, which reached a plateau within the first weeks post-injury (Fig. 2J and K). The co-occurrence of both pro- and anti-inflammatory molecules indicates that a substantial proportion of myeloid cells within the lesion core (mostly TMEM119+ cells) expressed an intermediate activation phenotype (Fig. 4K–P).

Within the lesion rim, the expression of the pro-inflammatory markers (i) was mostly detected on TMEM119+ myeloid cells (microglia); and (ii) peaked within the first 3 weeks (stage II) (Figs 3D–I, 4G and I). Contrary to the lesions core, the prominent pro-inflammatory patterns had not fully subsided at stage IV. Expression of CD163 was significantly elevated during the first weeks (stage II, P = 0.002; stage III, P = 0.032) (Figs 3J, 4Q and R). In contrast to CD163 and expression patterns observed in the lesion core, expression of the anti-inflammatory marker CD206 solely increased to non-significant extents at stage II and was rapidly lost thereafter. Notably, while 26% of Iba-1+ cells in the lesion core expressed CD206 at stage III, only 0.2% of Iba-1+ cells in the rim stained positive for CD206 (Fig. 4K–P). Iron-loaded macrophages were detected to a variable extent in the core and rim after 3 days (Figs 2L, 3L, 4S and T). Overall, our data show a time-dependent activation of microglia and macrophages, with a predominant pro-inflammatory expression pattern accentuated within the lesion rim and an intermediate pro- and anti-inflammatory phenotype within the lesion core.

**Cytokine expression patterns**

For the analysis of pro- and anti-inflammatory cytokine patterns, we investigated the expression of IL-1β and IL-18 as well as IL-10 and TGF-β, respectively, in two representative cases of each SCI stage as well as in control cases (Fig. 5). IL-1β and IL-10 immunoreactivity in myeloid cells was neither detectable in traumatic SCI nor control cases (data not shown). IL-18 was strongly expressed in microglia and macrophages in the lesion rim and core, respectively, throughout all SCI stages (Fig. 5A, B, F and G). Double labelling of IL-18 with the anti-inflammatory marker CD206 did not show any co-expression. (Fig. 5H and I); in SCI cases with particularly pronounced CD206 immunoreactivity, IL-18 expression was markedly reduced (Fig. 5I). TGF-β expression was only detected in astrocytes within the rim of lesion stages II and III and was absent in myeloid cells (Fig. 5C–E).

**T cells, B cells, plasma cells and complement deposition**

CD3+ T cells gradually infiltrated the lesion core over time (Fig. 6A, F and G). At the earliest time points in the lesion
Figure 4 Loss of ‘homeostatic’ microglia within the lesion core and rim. Segregation of infiltrating monocytes towards the core while microglia persist within the rim. Distinct and faceted microglia and macrophage response to human traumatic SCI (A). Almost all microglia are double positive for Iba-1 (green) and TMEM119 (red) as well as (B) co-express P2RY12 (green) and TMEM119 (red) in the spinal cord of non-traumatic controls. (C) The rim of the lesions contains high numbers of Iba-1 (green) TMEM119 (red) double-positive cells, while (D) TMEM119+ (red) cells are massively reduced in the lesion core. Likewise, (E) sparse TMEM119 (red) P2RY12 (green) double-positive cells are...
core, T cells mainly located to perivascular spaces (Fig. 6F), while scattered parenchymal CD3+ T cells were detected at stages II and III and declined thereafter. T cells were not encountered close to neurons or oligodendrocytes but were diffusely distributed. The group of CD3+ T cells was mainly composed of CD8+ cells and only few CD4+ cells (Fig. 6B, H and I). In the lesion rim, the majority of T cells accumulated in the perivascular space, while only a low number of T cells was truly dispersed in the parenchyma. Overall, numbers of CD8+ T cells remained elevated until the chronic stage IV.

In most SCI cases, B cells were hardly present; if any, they were mainly found in perivascular spaces intermingled with other perivascular cells (Fig. 6C, D, J and K). Only in two cases, we observed a pronounced infiltration of CD79a+ B cells and CD138+ IgG+-producing plasma cells into the spinal cord parenchyma (Fig. 6L–N). In these two cases as well as five other patients (total n of SCI cohort = 22), we also observed deposition of activated complement (C9neo antigen) in the area of tissue necrosis (Fig. 6O). Our data show a predominance of CD8+ T cells in SCI and, at least in single cases, an infiltration of B cells.

Oxidative injury

In control spinal cords, immunoreactivity for the oxidized phospholipid marker e06 was only observed in lipofuscin granules (Fig. 7C). After SCI, e06 was detected either as lipofuscin or was diffusely distributed in the cytoplasm of neurons as well as dendrites and axons (Fig. 7D and E). E06+ neurons often presented with an injured phenotype such as axonal beading (Fig. 7J), an irregularly shaped plasma membrane (Fig. 7K) or central chromatolysis (Fig. 7L). Oxidized phospholipids readily accumulated within the lesion core and peaked within the first 3 weeks post-SCI (Fig. 7A). Thereafter, e06 immunoreactivity readily declined to control levels. The e06 immunoreactivity in the lesion core positively correlated with the appearance of granulocytes (R = 0.426, P = 0.048). In the lesion rim, oxidized phospholipids were most abundant in early stage II (maximum at Day 4: 2.71% area); contrary to the lesion core, e06+ oxidized phospholipid immunoreactivity remained significantly elevated even at the chronic stage IV (P = 0.032) (Fig. 7B and E–H). Our data suggest that oxidative injury has a long-term contribution to protracted injury and neurodegeneration after SCI.

Discussion

We here provide the first systematic characterization of spatial and temporal patterns of innate and adaptive immune responses as well as oxidative tissue injury after traumatic SCI in a large cohort of patients (n = 22). Our study identified inflammatory patterns evolving differently within the lesion core versus the surrounding rim. The core was characterized by and infiltration of blood-derived monocytes/macrophages adapting an intermediate pro- and anti-inflammatory phenotype, while locally proliferating pro-inflammatory microglia dominated within the rim. Tissue infiltrating T cells were mainly identified as CD8+ cells. In a small subpopulation of SCI patients, we identify parenchymal B cells and plasma cells as well; further, parts of the SCI cohort displayed activated complement deposits in areas of tissue necrosis. We provide evidence that oxidative injury occurs not only in the acute phase but also even months and to 1.5 years after the impact.

Neutrophils are temporally restricted to the acute or early-subacute spinal cord injury phase

PMNs constitute the first immune cell population, which accumulated in traumatic SCI lesions within the first 72 h and were rapidly cleared thereafter. PMNs assembled within the lesion core in areas of haemorrhagic transformation and subsequent haemorrhagic necrosis. This is in contrast to ischaemic CNS injury, in which PMNs remain mostly confined to the boundaries of the neurovascular unit and do not actively infiltrate the lesioned parenchyma (Enzmann et al., 2013; Zrzavy et al., 2018). For SCI, it still remains unclear whether PMNs actively infiltrate the tissue because of chemotactic cues or are passively shuttled into the tissue along with extravasating blood. Generally, PMN infiltration oftentimes leads to subsequent cytotoxic tissue damage due to excessive cytokine and prostaglandin release, oxidative burst and release of toxic granules (Bao et al., 2004, 2008; Dinkel et al., 2004; Gris et al., 2004; Nguyen et al., 2007). Similarly, we observed within our SCI cohort a significant association between the presence of PMNs and the detection of e06+ oxidative neuronal injury in the lesion core. Taken

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**Figure 4 Continued**

still detectable in the rim, while (F) they are completely lost in the lesion core. In the lesion rim, the majority of microglia (TMEM119+ cells; red), expresses pro-inflammatory markers e.g. (G) p22phox (blue) (stage II) or (l) the phagocytosis-associated marker CD68 (blue); within the lesion core, hardly any myeloid cells expressing pro-inflammatory markers, e.g. (H) p22phox, co-express the microglia marker TMEM119. (J) Many microglia (TMEM119+; red) within the rim display nuclear co-expression of the proliferation marker proliferating cell nuclear antigen (PCNA; blue). (K) An example of an SCI lesion; within the core (left side), numerous pro-inflammatory (p22phox; blue) and anti-inflammatory (CD206; red) double-positive macrophages are present, whereas pro-inflammatory activation predominates within the rim (right side). (L and M) Higher magnification of lesion (L) core and (M) rim presented in K. (N) Same lesion presented in K showing sparse TMEM119 and CD206 double-positive cells within the lesion core (left side), whereas in the rim (right cells), CD206+ cells are hardly present and TMEM119 single-positive cells prevail. (O and P) Higher magnification of lesion (O) core and (P) rim presented in N. (Q) In the core of stage II lesions, numerous CD163+ macrophages are present; similarly, CD163+ microglia are observed in the rim (R). Low numbers of TBB+ cells were found in the core (S) as well as in the lesion rim (T). Scale bars = 25 µm. Ctl = Control; C = Core; I–IV = stages; R = Rim.
together, our data indicate that the early inflammatory injury after traumatic SCI, which is accompanied by a disturbed neurovascular unit and a possible developing systemic coagulopathy (Maegele et al., 2017), substantially differs from ischemic injury, particularly since the neurovascular unit evidently fails as a selective barrier for PMN infiltration after SCI.

**Formation of two myeloid cell compartments after spinal cord injury**

A crucial question is to what extent CNS-resident microglia and blood-derived myeloid cells contribute to the evolution further lesions (David and Kroner, 2011). Applying TMEM119, which has recently evolved as a marker exclusively expressed by microglia, we demonstrate that microglia were rapidly lost in the lesion core, while Iba-1+ TMEM119+ cells increased in this area. In contrast, within the rim a substantial proportion of Iba-1+ cells were derived from proliferating microglia cells in a similar percentage previously reported in mice (Greenhalgh and David, 2014).

This spatial segregation of different myeloid cell types mirrors exactly earlier reports studying bone marrow chimeric models (Popovich and Hickey, 2001). Infiltrating monocytes/macrophages have been suggested to stimulate remyelination (Kotter et al., 2003; Miron and Franklin, 2014), while on the contrary an overall detrimental net effect of monocyte infiltration into SCI lesions on the recovery of the locomotor system has been demonstrated earlier (Popovich et al., 1999). The net effect of microglia on neurological recovery remains unknown and is currently being investigated in pharmacological microglia ablation models. Of note, microglia deactivation resulted in the propagation of neurodegeneration after human CNS trauma suggestive of an at least partly protective role of microglia activation (Scott et al., 2018).

After experimental SCI, polarization of myeloid cells towards an M2 phenotype has been associated with enhanced axonal outgrowth and improved recovery by attenuating inflammation (Kigerl et al., 2009; Nakajima et al., 2012; Kroner et al., 2014). Generally, the M1/M2 paradigm may have to be dismissed in favour of a gradient of phenotypes between the both extremes M1 to M2. Particularly, the
Figure 6 Moderate T cell and sparse B cell infiltration into the spinal cord after human traumatic SCI. Profile of total lymphocytes numbers populating the injured SCI over time. (A–E) Quantitation of the expression of the T cell markers CD3 (A) and CD8 (B), the B cell markers CD20 (C) and CD79a (D) and the plasma cell marker CD138 (E) in SCI lesions at different lesion stages (I–IV) compared with non-traumatic control tissue. Data represent numbers of cells/mm². *p < 0.05; **p < 0.01; ***p < 0.001. (F) T cells were scarce, mostly perivascular, when observed in control patients. (G–I) Infiltrating CD3⁺ cells (G) in SCI are mainly MHC class I-restricted CD8⁺ cells (H), whereas only scattered CD4⁺ cells (I) were present in the injured neuropil. (J and K) Compared to T cells, single numbers of B cells mostly confined to perivascular areas were observed. (L–N) CD138⁺ plasma cells in a case of SCI was observed, which were identified as IgG positive plasma cells though double-labelling (N). (O) C9neo complement deposition of the same SCI case as in L–N. Scale bar = 25 μm.
characterization of phenotypes should include an extended panel of markers, since some traditional markers are not necessarily specific for the classical M1 or M2 phenotypes. For example, CD163, the haemoglobin-haptoglobin scavenger receptor, had originally been defined as an M2 marker but was later found to be involved in other pathways as well (Van Gorp et al., 2010). Furthermore, microglia in human disease conditions display mostly intermediate M1/M2 activation patterns as judged by the concomitant expression of pro- and anti-inflammatory markers (Vogel et al., 2013; Koepke et al., 2013). 

Figure 7 Oxidative injury after SCI affects spinal neurons. Presence of oxidized phospholipids (e06 immunoreactivity) in neurons has been associated with beading and fragmentation of cell processes in the human CNS (Fischer et al., 2012) and occurs after SCI. (A and B) Percentage of positive area covered by e06 immunoreactivity within the lesion core (A) and lesion rim (B). (C) Immunoreactivity of e06 in lipofuscin granules in non-traumatic control spinal cords. (D) Few e06⁺ axonal bulbs within the lesion core during the first 3 days post-SCI (stage I). (E) Several e06⁺ axonal bulbs within the rim of chronic spinal cord lesions (stage IV). (F) At stage II, many APP⁺ axonal bulbs are present in the lesion rim, where oxidized phospholipids also accumulate (G). (H) Co-accumulation of oxidized phospholipids and APP in ballooned axonal spheroids and retraction bulbs. (I) Macrophages with granular cytoplasmic reactivity for oxidized phospholipids in the SCI core. (J) Intense e06 immunoreactivity in a neuron showing signs of degeneration and beading/fragmentation of cell processes. (K) Massive accumulation of oxidized phospholipids (e06⁺) in a degenerating neuron surrounded from and in contact with pro-inflammatory p22phox⁺ microglia (blue). (L) e06⁺ neuron undergoing central chromatolysis in contact with activated pro-inflammatory p22phox⁺ microglia. Scale bar = 25 μm.
Peereen et al., 2015; Zrzavy et al., 2017, 2018, 2019). Finally, certain microglia functions, such as phagocytosis, had originally been defined as pro-inflammatory and disease promoting, but turned out afterwards to be essential in limiting the progression of tissue injury and in promoting tissue repair (Kotter et al., 2005; Ulrich et al., 2017). Thus, instead of strictly categorizing microglia and macrophage differentiation into M1 and M2, it seems more relevant to specifically define their functional properties. Here, we demonstrate that the homeostatic marker P2RY12 was promptly downregulated in a longstanding manner after SCI. Simultaneously, microglia/macrophages responded to the acute traumatic injury (Fleming et al., 2006; Beck et al., 2010; Krasmann et al., 2017) with an upregulated expression of molecules involved in oxygen species generation (iNOS, p22phox), antigen presentation (MHC II), phagocytosis (enlarged CD68+ lysosomes) and increased levels of IL-18. This inflammatory pattern endures the early phases (stage I and II) and persists during the late sub-acute (stage III) and is evident even until chronic stages (stage IV) reflecting a chronic inflammatory state and possibly exacerbating of tissue injury. Prominent expression of CD163 in macrophages suggests that these cells are instrumental in the removal of haemoglobin from the lesion site; this is further supported by the detection of enhanced iron accumulation in a subset of macrophages/microglia. Accumulation of iron in oligodendrocytes, other glial cells and neurons, which in general occurs in the vicinity of haemorrhagic lesions in the brain (Hametner et al., 2013; Glushakova et al., 2014), was sparse or even absent in SCI lesions. Noteworthy, detection of interleukins via immunohistochemistry in archival formalin-fixed paraffin-embedded tissue is known to be challenging. However, the here applied panel of pro/anti-inflammatory cytokines has already been successfully applied in inflammatory CNS conditions (Machado-Santos et al., 2018; Trösch et al., 2019), which is why we are confident that the absence of IL-1β and IL-10 expression in myeloid cells is indeed representative.

**Oxidative activation and oxidative injury after human spinal cord injury**

Neuropathological studies have previously shown neuronal oxidative injury in inflammatory brain lesions in humans (Haider et al., 2011; Fischer et al., 2013). Experimental SCI studies indicate that reactive oxygen species produced by myeloid cells contribute to protracted tissue damage (Bao et al., 2004; Pajoohesh-Ganji and Byrnes, 2011). Here, we analysed the intracellular deposition of oxidative phospholipids via e06 immunohistochemistry as an established marker for oxidative injury (Haider et al., 2011). This was prominently seen in the rim surrounding the necrotic core of the lesion. This indicates that oxidative injury is a major contributor of tissue damage, not only in the initial stages of injury, but even progressing during the following months (Hausmann, 2003; Bastani et al., 2012).

**Lymphocytes are present during tissue remodelling and sustained injury**

Lymphocytes have previously been described to populate the lesion site in comparatively low numbers after experimental SCI (Sroga et al., 2003). Similarly, we only detected moderate numbers in our SCI cohort. A more detailed characterization revealed CD8+ T cells as the dominant lymphocyte population within spinal cord lesions. The dominance of CD8+ T cells is, however, not specific for SCI, but has already been seen in other inflammatory or neurodegenerative diseases of the human CNS. Even in the non-diseased brain the sparse parenchymal T cells compose of CD8+ over CD4+ cells. (van Nierop et al., 2017; Machado-Santos et al., 2018). Whether these cells play an active role in human SCI lesions or are passively recruited to the site of tissue damage remains unresolved.

Mice who are devoid of B cells showed better recovery after experimentally induced SCI (Ankeny et al., 2009). Moreover, the production of auto-antibodies has been demonstrated in experimental models; injection of IgG fractions from SCI animals into naïve spinal cords subsequently triggered considerable neurotoxicity (Ankeny et al., 2009). In humans, auto-antibodies directed against CNS antigens have been found in the serum after SCI (Hayes et al., 2002; Zajarias-Fainsod et al., 2012; for a review see Schwab et al., 2014). In a previous study, B cells could not be detected in the affected areas after SCI in humans (Fleming et al., 2006). In our study cohort comprising 22 SCI cases, we observed CD79a+ B cells and CD138+ plasma cells in the lesioned spinal cords in two patients. CD138+ plasma cells were IgG+ (Fig. 6L) indicating active antibody synthesis. Although B cells and plasma cells were very rare in the majority of SCI lesions, we nevertheless provide neuropathological evidence that, at least in a subpopulation of SCI patients: (i) B cells infiltrate the lesioned cord; (ii) convert into plasma cells; and (iii) begin to synthesize antibodies. Furthermore, complement deposition was observed within those and three additionally cases. Thus, B cell- and antibody-mediated neurotoxic immune mechanisms may contribute to tissue injury in a subset of SCI patients.

Several factors should be considered in the interpretation of our data. First, we do not have data on any treatment of our patients with methylprednisolone in the acute phase of injury. Methylprednisolone might have impacted on the inflammatory kinetics leading to a shift of the inflammatory response and potentially impacting on oxidative injury (Hall, 1993). If anything, it might have led to an attenuation of the acute inflammatory phase and thus, to an underestimation of the observed inflammatory neuropathology evident in our study cohort. As methylprednisolone is still occasionally applied to SCI patients, our findings might...
represent the actual treatment situation and the actual pathology in ‘partly treated–partly untreated’ SCI patients very well. As another limitation of the study, it was not possible to determine in our SCI cohort, whether the degree of inflammation within the lesions was influenced by the severity of SCI as measured by the Asia Impairment Scale (AIS). A difference in AIS values within the four groups (stages I–IV) may modify the quantitative assessments but not the qualitative findings as such. We therefore refrained from further correlative analyses and focused on qualitative assessments. It should be noted that earlier reports (Fleming et al., 2006) do not provide this information either.

**Conclusion**

Our data revise and amend the chronological order of pro- and anti-inflammatory cellular mediators upon traumatic SCI in humans. In this immunohistochemical study, we provide evidence for differing myeloid responses developing within the lesion core and the lesion rim post-SCI. The core mainly harbours blood-derived monocytes/macrophages, which further adopt an intermediate pro- and anti-inflammatory phenotype, while microglia within the rim locally proliferate and show a predominantly pro-inflammatory profile. Similarly, neuronal oxidative injury in the lesion core declines within months post-SCI, while it persisted in the lesion rims until the latest time points analysed (1.5 years post-SCI). The here-described long-lasting preservation of oxidative tissue injury might be a substantial contributor to (i) an impaired response to rehabilitation; (ii) overall failure of recovery; or (iii) delayed loss of recovered function. The different inflammatory cellular composition and activation in the lesion core compared with its rim results in distinct inflammatory milieus of relevance for topical cell transplantation, but also neuroprotective, or immunomodulatory interventions in SCI patients.

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**Competing interests**

The authors report no competing interests.

**Supplementary material**

Supplementary material is available at Brain online.

**References**

Ahuja CS, Wilson JR, Nori S, Kotter MR, Druschel C, Curt A, et al. Traumatic spinal cord injury. Nat Rev Dis Primers 2017; 3: 1–21.

Ankeny DP, Guan Z, Popovich PG. B cells produce pathogenic antibodies and impair recovery after spinal cord injury in mice. J Clin Invest 2009; 119: 2990–9.

Ankeny DP, Lucin KM, Sanders VM, MaGaughy VM, Popovich PG. Spinal cord injury triggers systemic autoimmunity: evidence for chronic B lymphocyte activation and lupus-like autoantibody synthesis. J Neurochem 2006; 99: 1073–87.

Bao F, Chen Y, Dekaban GA, Weaver LC. Early anti-inflammatory treatment reduces lipid peroxidation and protein nitration after spinal cord injury in rats. J Neurochem 2004; 88: 1335–44.

Bao F, Chen Y, Schneider KA, Weaver LC. An integrin inhibiting molecule decreases oxidative damage and improves neurological function after spinal cord injury. Exp Neurol 2008; 214: 160–7.

Bastani NE, Kostovski E, Sakhi AK, Karlsen A, Carlsen MH, Hjelmes N, et al. Reduced antioxidant defense and increased oxidative stress in spinal cord injured patients. Arch Phys Med Rehabil 2012; 93: 2223–8.e2.

Bauer J, Lassmann H. Neuropathological techniques to investigate central nervous system sections in multiple sclerosis. Methods Mol Biol 2016; 1304: 211–29.

Beck KD, Nguyen HX, Galvan MD, Salazar DL, Woodruff TM, Anderson AJ. Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. Brain 2010; 133: 433–47.

Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, et al. New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci USA 2016; 113: E1738–46.

Bien CG, Vincent A, Barnett MH, Becker AJ, Blümcke I, Graus F, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. Brain 2012; 135: 1622–38.

Butovsky O, Jedrychowski MP, Moore CS, Civic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat Neurosci 2014; 17: 131–43.

David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. Nat Rev Neurosci 2011; 12: 388–99.

David S, Lopez-Vales R, Wee Yong V. Harmful and beneficial effects of inflammation after spinal cord injury: potential therapeutic implications. Handb Clin Neurol 2012; 109: 485–502.

Dinkel K, Dhabhar FS, Sapolsky RM. Neurotoxic effects of polymorphonuclear granulocytes on hippocampal primary cultures. Proc Natl Acad Sci USA 2004; 101: 331–6.
Inflammation and oxidative injury in SCI

Kigerl KA, Genzel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci 2009; 29: 13435–44.

Kirshblum S, Mills S, McKinley W, Tulsky D. Late neurologic recovery after traumatic spinal cord injury. Arch Phys Med Rehabil 2004; 85: 1811–7.

Kotter MR, Zhao C, van Rooijen N, Franklin RJ. Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. Neurobiol Dis 2005; 18: 166–75.

Krasemann S, Madore C, Cialle R, Baufeld C, Calcagno N, El Fatimy R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity 2017; 47: 566–81.e9.

Kroner A, Greenhalgh AD, Zarruk JG, dos Santos RP, Gaestel M, David S. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. Neuroreport 2014; 18: 998–1002.

Miron VE, Franklin RJ. Macrophages and CNS remyelination. J Neurochem 2014; 130: 165–71.

Nakajima H, Uchida K, Guerrero AR, Watanabe S, Sugita D, Takeura N, et al. Transplantation of mesenchymal stem cells promotes an alternative pathway of macrophage activation and functional recovery after spinal cord injury. J Neurotrauma 2012; 29: 1614–25.

Nguyen HX, O'Barr TJ, Anderson AJ. Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. J Neurochem 2007; 100: 900–12.

Ort MB, Gensel JC. Spinal cord injury scarring and inflammation: therapies targeting glial and inflammatory responses. Neurotherapeutics 2018; 15: 541–53.

Pajoohesh-Ganji A, Byrnes KR. Novel neuroinflammatory targets in the chronically injured spinal cord. Neurotherapeutics 2011; 8: 195–205.

Pfefferen LA, Vogel DY, Ummemuth K, Breur M, Heijnen PD, Gerritsen WH, et al. Activation status of human microglia is dependent on lesion formation stage and remyelination in multiple sclerosis. J Neuropathol Exp Neurol 2015; 74: 48–63.

Popovich PG, Guan Z, Wei P, Huitinga I, van Rooijen N, Stokes BT. Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. Exp Neurol 1999; 158: 351–65.

Popovich PG, Hickey WF. Bone marrow chimeric rats reveal the unique distribution of resident and recruited macrophages in the contused rat spinal cord. J Neuropathol Exp Neurol 2001; 60: 676–85.

Pruss H, Kopp MA, Brommer B, Gatzemeier N, Laginha I, Dirnagl U, et al. Non-resolving aspects of acute inflammation after spinal cord injury (SCI): indices and resolution plateau. Brain 2011; 21: 652–60.

Raofer LM, Ramer MS, Bradbury EJ. Restoring function after spinal cord injury: towards clinical translation of experimental strategies. Lancet Neurol 2014; 13: 1241–56.

Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 2016; 19: 987–91.
