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PLoS MEDICINE

Infiltrating Blood-Derived Macrophages Are Vital Cells Playing an Anti-inflammatory Role in Recovery from Spinal Cord Injury in Mice

Ravid Shechter^{1,3}, Anat London^{1,3}, Chen Varol^{2,3}, Catarina Raposo¹, Melania Cusimano³, Gili Yovel¹, Asya Rolls¹, Matthias Mack⁴, Stefano Pluchino³, Gianvito Martino³, Steffen Jung^{2,3*}, Michal Schwartz^{1,3*}

1 Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel, **2** Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel, **3** Neuroimmunology Unit, DIBIT and Institute of Experimental Neurology (INSPE), San Raffaele Scientific Institute, Milan, Italy, **4** Department of Internal Medicine, University of Regensburg, Regensburg, Germany

Thomas HAIDER
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Background

Spinal Cord Injury

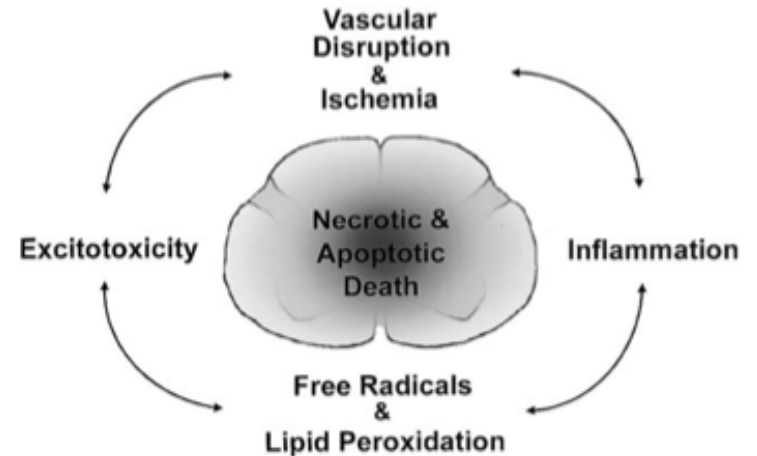
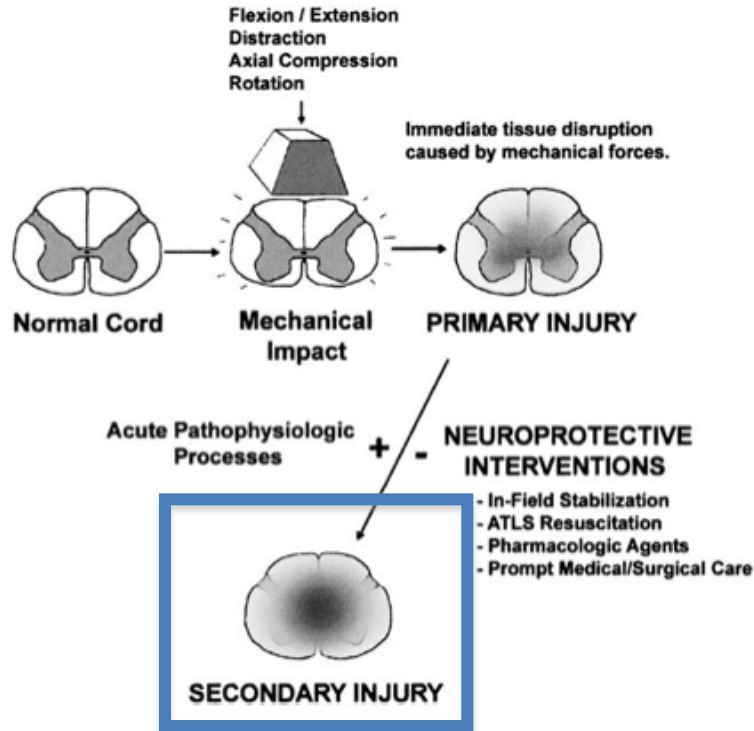
- 50 per 1 million annually
- Mainly young patients
- 50% - total loss of motor function
- 2/3 - cervical spinal cord injury
- 70% - suffer from chronic pain

Tator CH, Fehlings MG. J Neurosurg. 1991; **75**(1): 15-26.

Ackery A, Tator C, Krassioukov A. Journal of neurotrauma. 2004; **21**(10): 1355-70.

Vienna, 2014

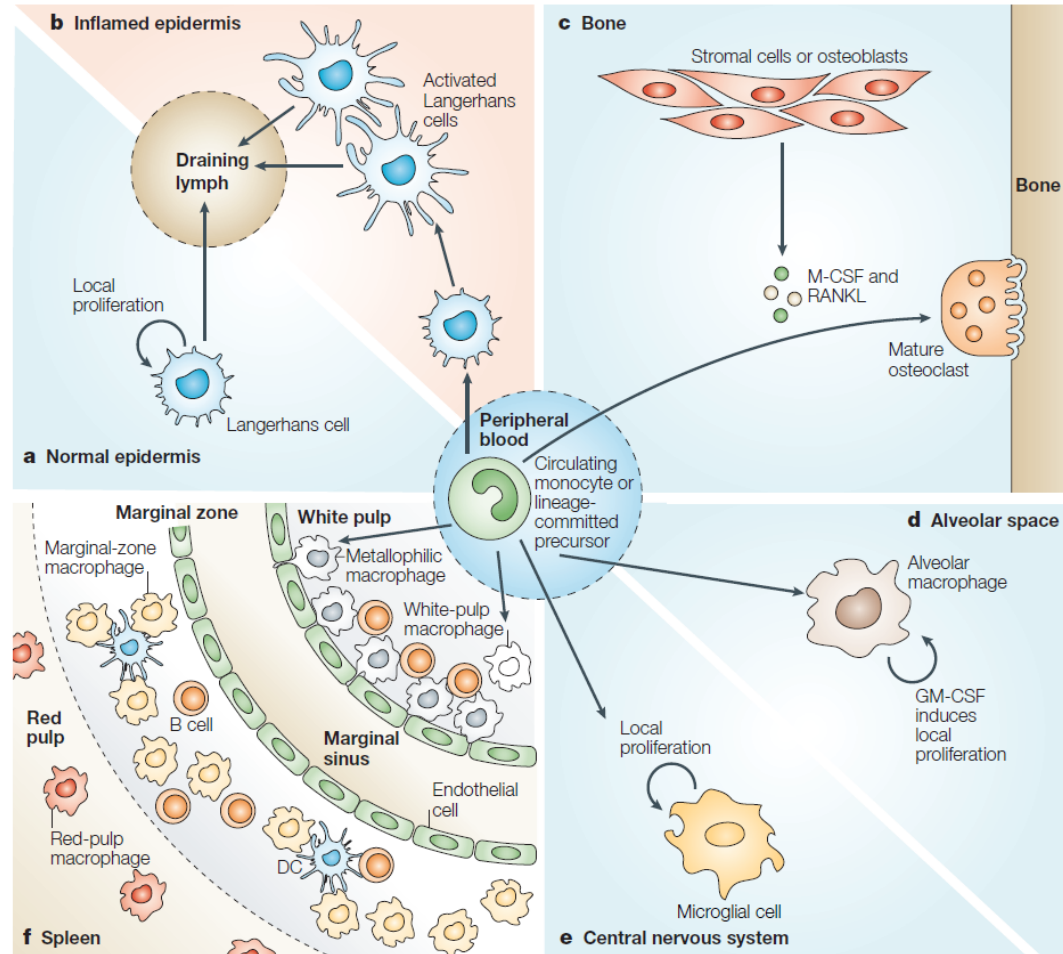
Background



Background

Monocytes

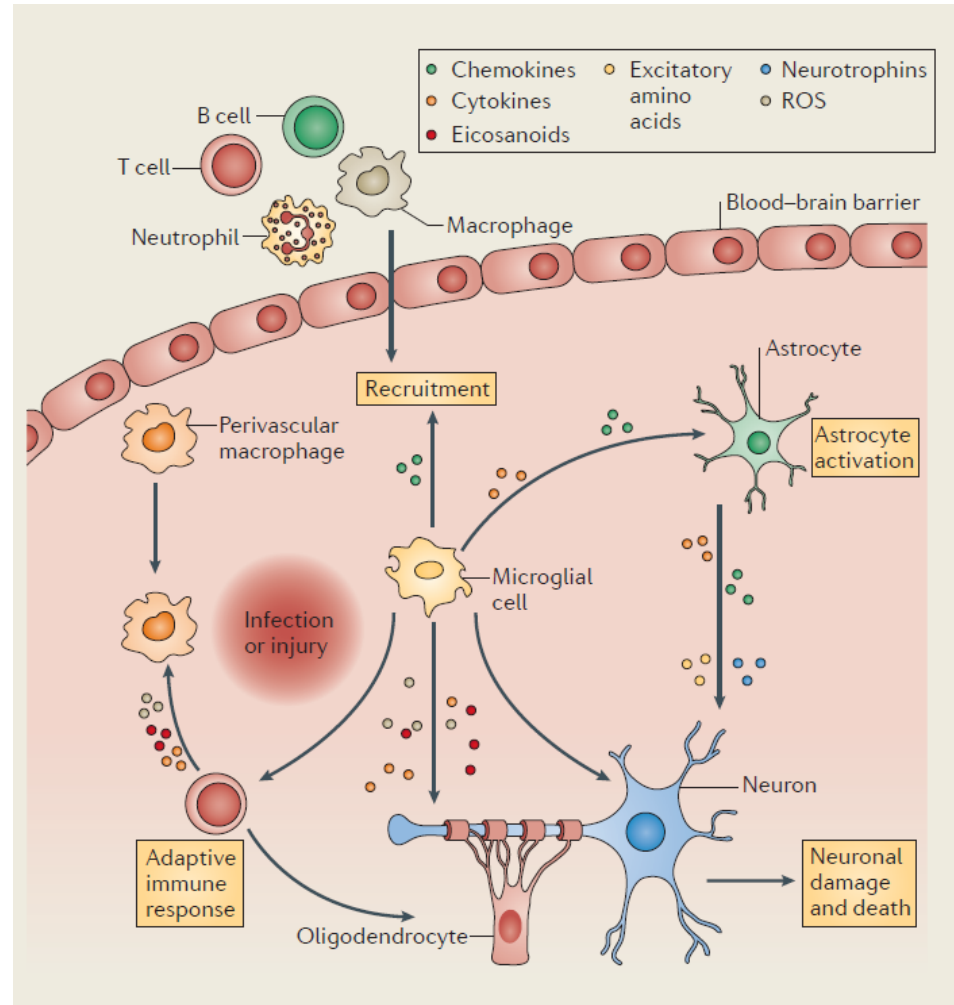
- Myeloid Progenitor
- 5-10% of PBMCs
- Pro-inflammatory, metabolic and immune stimuli



Background

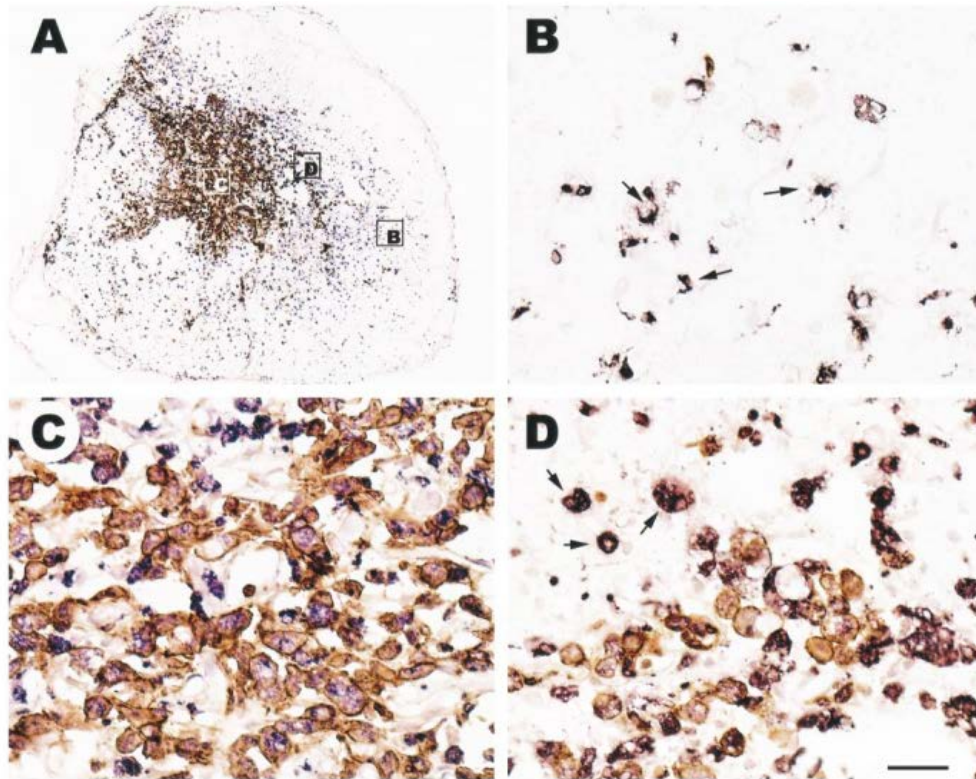
Microglia

- Myeloid progenitor enter CNS during development
- Histiocytes of the CNS
- Density shows local differences



Background

Microglia and Macrophages in SCI

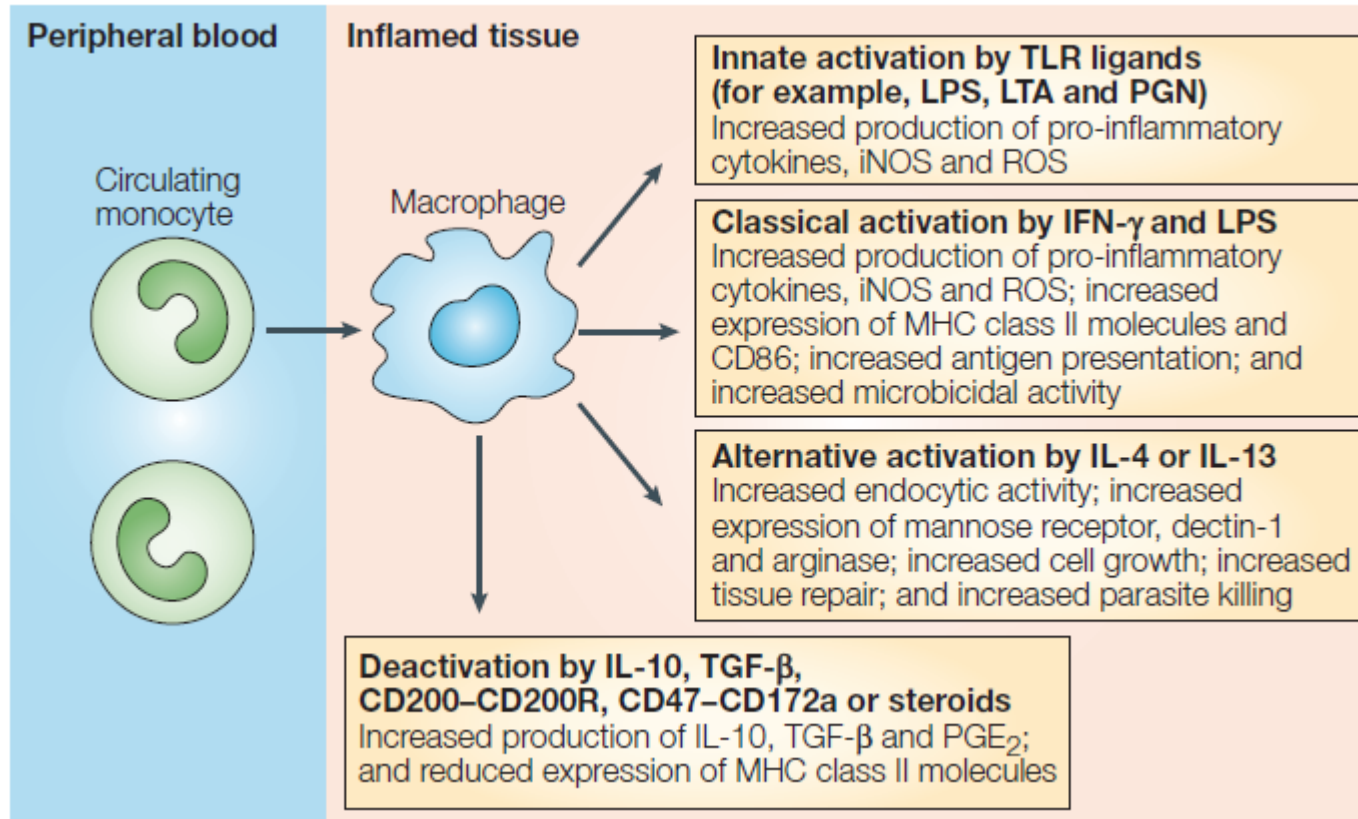


Background

Macrophages

- Past ten years: Heterogenous population
- Pro- AND Anti-Inflammatory activities
- Subsets

Background



Background

Troubleshooting

- Microglia and macrophages cannot be distinguished by standard immunohistochemistry
- Use of chimeric models

Background

The aim of the study...

... was to investigate the role of monocyte derived macrophages in spinal cord injury in a murine model.

Methods

- Murine SCI contusion model (T12, 200 kdynes)
- Seven types of C57BL/6J mice were used (wild-type and various knock-outs)
- BM radiation chimeras

Results

Results

1st Setting

- Wild-type C57BL/6J – contusion injury
- Adoptive transfer of labeled naïve monocytes
(Cx₃cr1^{GFP/+}, CD45.1)

Results

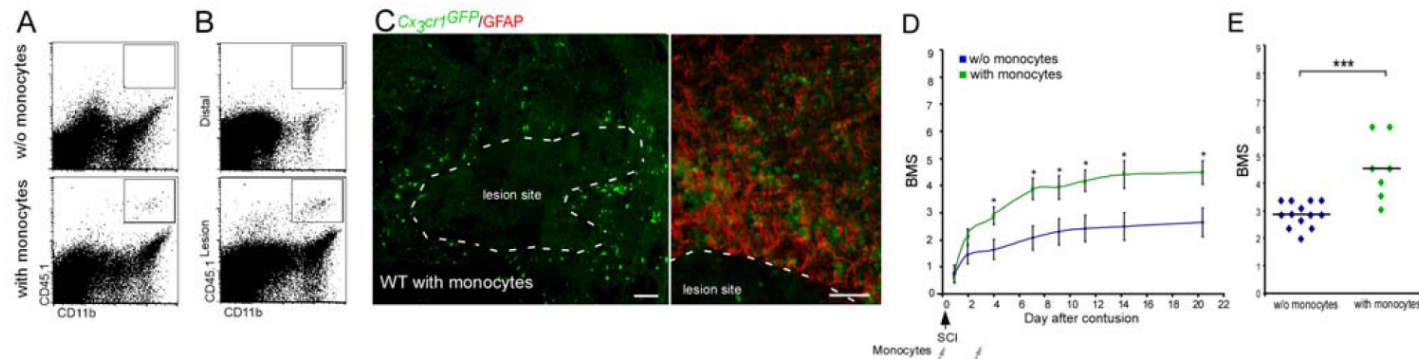


Figure 1. Monocyte-derived macrophages, spontaneously recruited to the injured spinal cord following the injury, promote functional recovery. Wild-type mice were subjected to SCI and received passive transfer (injected intravenously) of monocytes (CD45.1⁺ or *Cx₃cr1*^{GFP}) during the first week of recovery. (A–C) Spinal cords were excised 7 d after the injury and analyzed for the presence of infiltrating monocyte-derived MΦ. Flow cytometric analysis of (A) lesion area (4 mm segment) of injured spinal cord from mice treated with and without (w/o) adoptive transfer of monocytes (CD45.1⁺/CD11b⁺), indicating the arrival of graft-derived MΦ to the lesion area. (B) Flow cytometric analysis of lesion and distal areas (4 mm segment each) from injured spinal cords of adoptively transferred mice indicating the accumulation of the graft-derived MΦ (CD45.1⁺/CD11b⁺) mainly at the lesion and not at the distal areas (2,259 ± 431 engrafted cells per gram of tissue taken from lesion area [mean ± SE]). (C) Immunohistochemical analysis showing the adoptively transferred cells (*Cx₃cr1*^{GFP/+}; green) restricted to the margins of the lesion site, delineated by GFAP expression (red, right frame) (scale bar = 100 μm). (D) Similarly treated animals were followed for locomotor activity assessed according to the BMS (repeated measures ANOVA; $F_{[between\ groups]_{1,18}} = 16.7$; $p = 0.0007$). *y*-Axis error bar represents SE. (E) Mean locomotor score (BMS) of individual mice on d28 after spinal cord injury (Student's *t*-test; $t = -5.09$; $df = 15$; $p = 0.0001$), suggesting that increasing the pool of naïve monocytes by IV injection of wt mice following SCI enhanced recovery beyond spontaneous levels. The assessment of the functional outcome presented here is from one experiment representative of three independent experiments performed.
doi:10.1371/journal.pmed.1000113.g001

Results

2nd Setting

- ?



Results

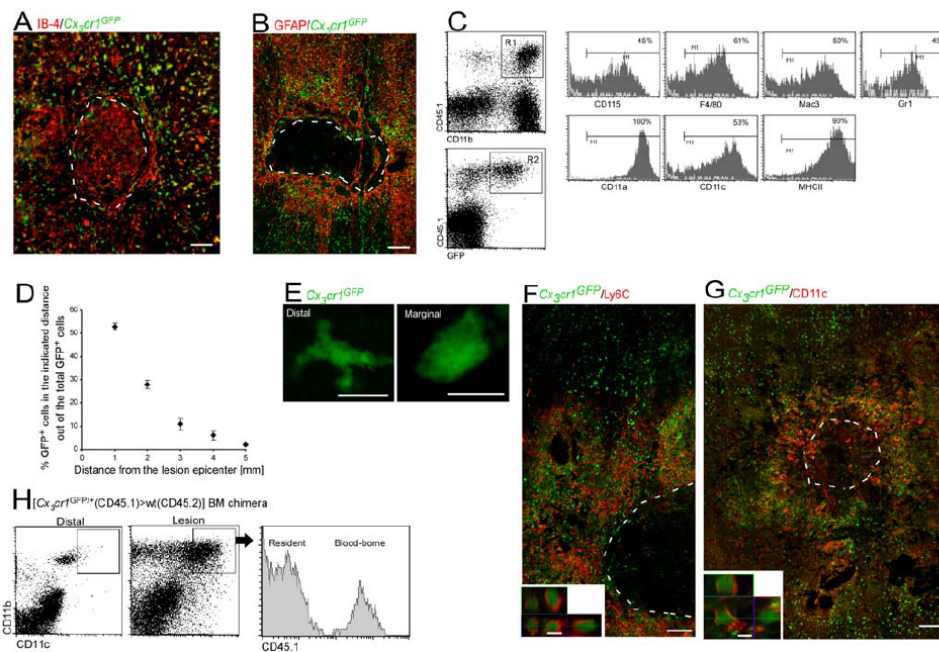


Figure 2. Monocyte-derived macrophages acquire a unique phenotype in close proximity to the lesion site. Chimeric mice were subjected to SCI and analyzed a week later for homing of cells. (A) Cells labeled for I8-4 (red), and GFP (green) at the lesion site of $[Cx3cr1^{GFP/+}] > wt$ BM chimeric mice. (B) Cells labeled for GFAP (red) and GFP (green), demonstrating that the infiltrating myeloid cells barely penetrate the lesion epicenter. (C) Representative flow cytometric analysis showing the extent of expression of various markers by the infiltrating myeloid cells ($CD11b^{+}/Cx3cr1^{GFP/+}/CD45.1^{-}$) in the injured spinal cords of $[Cx3cr1^{GFP/+}(CD45.1) > wt (CD45.2)]$ BM chimeras. The numbers above the bars refer to the percentage of the cells positive for the indicated marker out of the $R1 \times R2$ population (representing the infiltrating monocytes). The bars point to cells positive for the indicated marker (isotype control, gray line). (D) Spatial distribution map of monocyte-derived M Φ (GFP $^{+}$) at specific distances relative to the epicenter of the lesion site in $[Cx3cr1^{GFP/+}] > wt$ BM chimeras based on immunohistochemical analysis. (E) High magnification of GFP $^{+}$ cells (green) from distal and marginal areas of the lesion, demonstrating morphological differences. (F) Representative confocal micrograph of longitudinal sections from injured spinal cord of $[Cx3cr1^{GFP/+}] > wt$ BM chimeras, labeled for Ly6C (red), and infiltrating monocyte-derived M Φ by GFP (green). Lower panel: z-axis projection of a single cell. (G) Representative confocal micrograph of longitudinal sections of injured spinal cord, labeled for monocyte-derived M Φ by GFP (green) and CD11c (red). Lower panel: z-axis projection of single cell. (H) Flow cytometric analysis of distal and lesion spinal cord samples for CD11c expression on M Φ ($CD11b^{+}$ cells) in $[Cx3cr1^{GFP/+}(CD45.1) > wt (CD45.2)]$ BM chimeras. Note the higher incidence of $CD11c^{high}$ cells in the lesion sample. The histogram to the right is gated on $CD11b^{+}/CD11c^{high}$ cells at the lesion area, showing that both resident ($CD45.1^{-}$) and infiltrating ($CD45.1^{+}$) cells express CD11c. The dashed line demarcates the lesion site in (A), (B), (F), and (G), as determined by GFAP

Discussion

Discussion

- Emerging evidence of different monocyte/macrophage physiology in humans compared to rodent models

Thank you for your attention!