Impact of depletion of microglia/macrophages on regeneration after spinal cord injury

Igor Jakovčevski[1,2], Eckart Förster[2], Gebhard Reiss[1]

[1]Institut für Anatomie und Klinische Morphologie, Universität Witten/Herdecke, Witten, Germany; [2]Institut für Neuroanatomie und Molekulare Hirnforschung, Ruhr Universität Bochum, Bochum, Germany

ABSTRACT

Microglia/macrophages play important functional roles in regeneration after central nervous system injury. Infiltration of circulating macrophages and proliferation of resident microglia occur within minutes following spinal cord injury. Activated microglia/macrophages clear tissue debris, but activation over time may hamper repair. To study the role of these cells in regeneration after spinal cord injury we used CD11b-HSVTK (TK) transgenic mice, in which viral thymidine kinase activates ganciclovir toxicity in CD11b-expressing myeloid cells, including macrophages and microglia. A severe reduction in number of these cells was seen in TK versus wild-type littermate mice at 1 week and 5 weeks after injury, and numbers of Mac-2 expressing activated microglia/macrophages were almost completely reduced at these time points. One week after injury TK mice showed better locomotor recovery, but recovery was similar to wild-type mice as measured weekly up to 5 weeks thereafter. At 5 weeks after injury, numbers of axons at the lesion site did not differ between groups. Also, catecholaminergic innervation of spinal motoneurons was similar. However, cholinergic innervation was lower and glial scarring was increased in TK mice compared to wild-type mice. We conclude that reducing numbers of CD11b-expressing cells improves locomotor recovery in the early phase after spinal cord injury, but does not affect recovery in the following 4 weeks. These observations point to differences in outcomes of astrocytic and cholinergic responses under CD11b cell ablation, which are, however, not reflected in the locomotor parameters analyzed at 5 weeks after injury.

Figure 1. Scheme of the experimental protocol

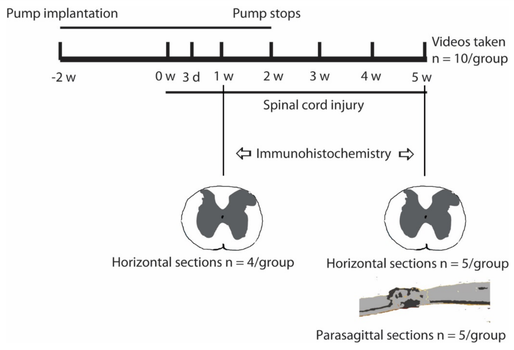

Figure 1. The osmotic pump with ganciclovir is implanted 2 weeks prior to spinal cord injury (-2 w). The pump releases ganciclovir for 4 weeks, i.e. until 2 weeks after injury (2 w). Video recordings for motor analysis are taken before pump implantation (-2 w), before spinal cord injury (0 w), and 3 days (3 d), 1, 2, 3, 4 and 5 weeks after injury. Five weeks after spinal cord injury the animals are sacrificed for histological analysis. Five mice/group were used for longitudinal, parasagittal sections, and another 5 for horizontal sections. Another group of animals (n = 4/group) was sacrificed at 1 week after injury for estimation of microglia ablation.

Figure 2. Numbers of microglia/macrophages in lumbar spinal cords of TK and WT mice 1 week and 5 weeks after injury

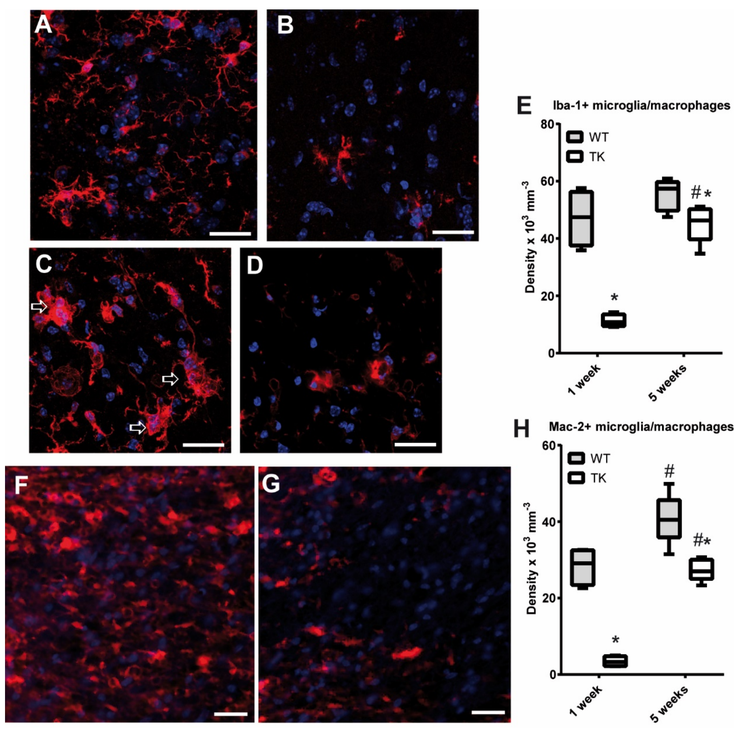

Figure 2. (**A-D**) Representative images from WT (**A,C**) and TK (**B,D**) gray (**A,B**) and white matter (**C,D**) of lumbar spinal cords immunostained for Iba-1, 1 week after injury. Arrows point at clumps of 3-5 cells. (**E**) Box-and-whiskers plots for number of Iba-1+ cells per volume. Asterisks indicate mean value significantly different from WT, hashtag indicates values significantly different from 1 week (two-way ANOVA with Holm-Sidak *post-hoc* test, $p < 0.05$). Scale bars: 20 µm. (**F,G**) Representative images from WT (**F**) and TK (**G**) lumbar spinal cords immunostained for Mac-2, 1 week after injury. (**H**) Box-and-whiskers plots for number of Mac-2+ cells per volume. Asterisks indicate mean value significantly different from WT, hashtag indicates values significantly different from 1 week (two-way ANOVA with Holm-Sidak *post-hoc* test, $p < 0.05$). Scale bars: 20 µm.

Figure 3. Time course and degree of functional recovery in TK and WT mice after spinal cord injury

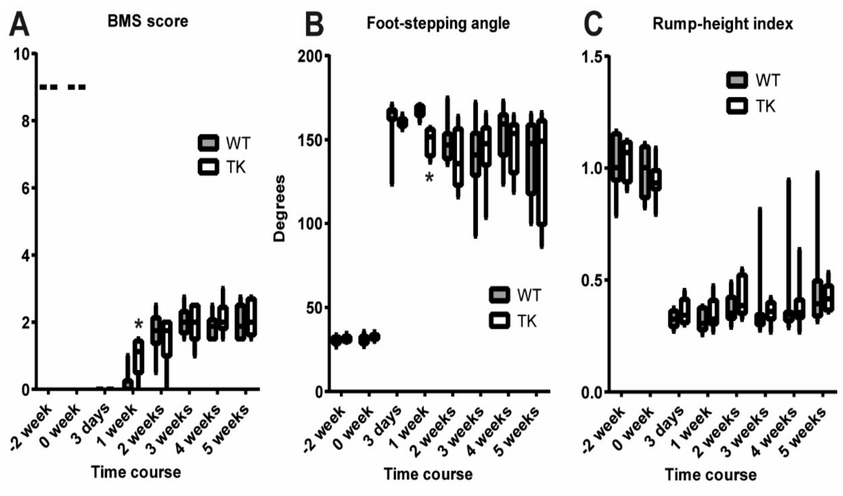

Figure 3. Box-and-whiskers plots for (**A**) BMS score, (**B**) foot-stepping angle and (**C**) rump-height index before (-2w) and 2 weeks after the placing of the Alzet pump (0w), and 3 days (3d), 1, 2, 3, 4 and 5 weeks (w) after spinal cord injury. Asterisks indicate significant differences between group mean values at a given time point (two-way ANOVA for repeated measures with Holm-Sidak *post-hoc* test, $p < 0.05$). (**D**) Box-and-whiskers plot for BMS score with added saline control infused TK and WT mice. Asterix indicates significant difference from other groups (two-way ANOVA for repeated measures with Holm-Sidak *post-hoc* test, $p < 0.05$).

Figure 4. Number of NF+ axons at the lesion site and TH+ axons 500 µm caudal to the lesion site

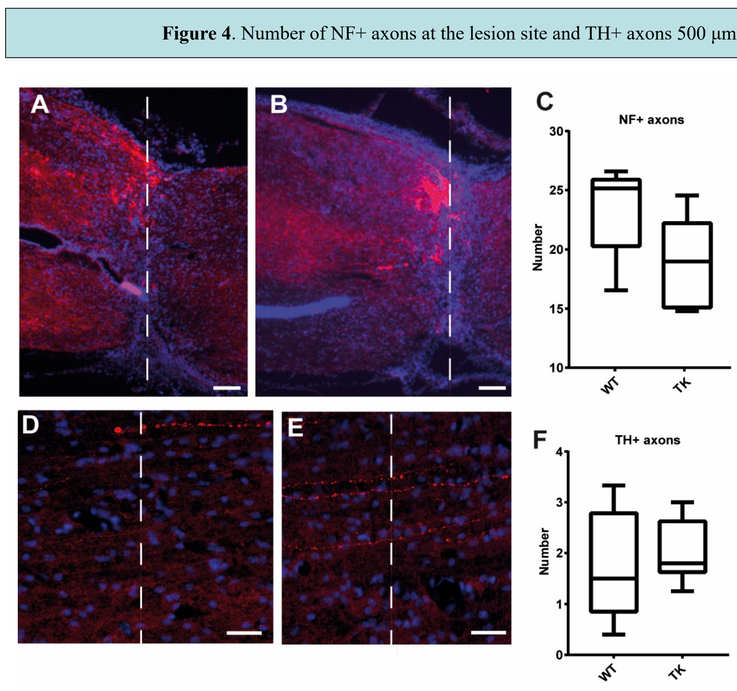

Figure 4. (**A**,**B**) Representative images of spinal cords immunostained for neurofilament 200 in (**A**) wild-type (WT) and (**B**) TK mice. Dashed line represents lesion center. (**C**) Box-and-whiskers plot for numbers of NF+ axons at the lesion center in WT and TK mice 5 weeks after injury (t-test, $p > 0.05$). (**D**,**E**) Representative images of spinal cords immunostained for tyrosine hydroxylase in WT (**D**) and TK (**E**) mice. Dashed line represents distance caudal to the lesion site where counting was performed. (**F**) Box-and-whiskers plot for numbers of TH+ axons 500 µm caudal to the lesion site in WT and TK mice 6 weeks after injury (t-test, $p > 0.05$). Scale bars: 100 µm.

Figure 5. The cell body area and cholinergic terminals on motoneurons in the lumbar spinal cord caudal to the lesion site 5 weeks after spinal cord injury

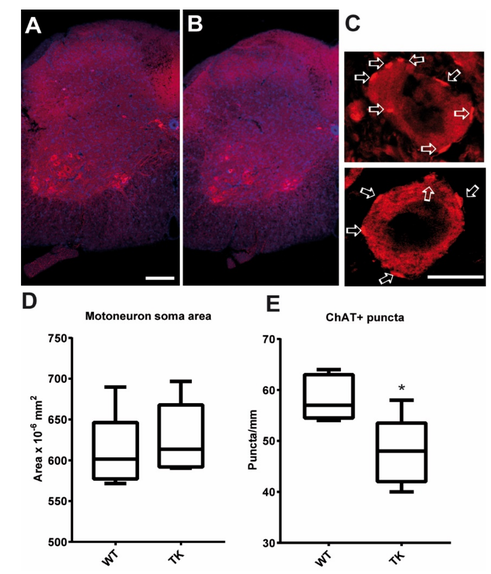

Figure 5. (**A**,**B**) Representative image of a wild-type (WT, A) and TK (B) spinal cord coronal hemisection immunostained for ChAT (red) and counterstained with bisbenzimide (blue). (**C**) Single ChAT+ motoneurons with arrows pointing to puncta from a WT (upper panel) and TK (lower panel) mouse. (**D**) Box-and-whiskers plot for cell body area of spinal motoneurons (t-test, $p > 0.05$). (**E**) Box-and-whiskers plot for ChAT+ terminals per unit length (mm) of motor neuron cell body perimeter. Higher numbers of cholinergic synapses are found on motoneurons of injured *WT than TK* mice. Asterisk indicates mean values significantly different from WT (t-test, $p < 0.05$). Scale bars: 200 µm (**A**,**B**) and 20 µm (**C**).

Figure 6. Density of GFAP+ astrocytes in the lumbar spinal cord of WT and TK mice 1 and 5 weeks and GFAP+ scar area 5 weeks after spinal cord injury

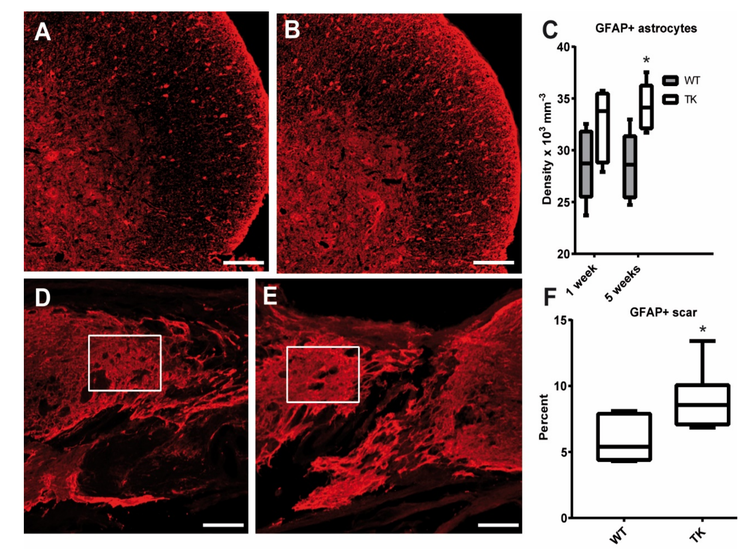

Figure 6. (**A**,**B**) Representative images of coronal sections of lumbar spinal cords immunostained for GFAP from WT (**A**) and *TK* (**B**) mice 5 weeks after injury. (**C**) Box-and-whiskers plots for numbers of GFAP+ cells per volume. Asterisk indicates mean values significantly different from WT (two-way ANOVA with Holm-Sidak *post-hoc* test, $p < 0.05$). (**D**,**E**) Representative images of parasagittal sections 5 weeks after injury immunostained for GFAP at the lesion site in WT (**D**) and TK (**E**) mice. Boxes depict areas taken for the analysis. (**F**) Box-and-whiskers plot for percentage of GFAP+ area at the lesion site. Asterisk indicates mean values significantly different from WT (t-test, $p < 0.05$). Scale bars: 100 µm (**A**,**B**) and 200 µm (**D**,**E**).

CONCLUSIONS

- We accomplished a severe reduction in number of microglia/macrophages in CD11b-HSVTK (TK) transgenic mice at 1 week and 5 weeks after injury.
- Numbers of Mac-2 expressing activated microglia/macrophages were almost completely reduced at 1 week after injury and remained severely depleted until 5 weeks.
- One week after injury TK mice showed better locomotor recovery, but recovery was similar to wild-type mice as measured weekly up to 5 weeks thereafter.
- At 5 weeks after injury, numbers of axons at the lesion site and catecholaminergic innervation of spinal motoneurons did not differ between groups.
- Cholinergic innervation of spinal motoneurons was lower and glial scarring was increased in TK mice compared to wild-type mice 5 weeks after injury.
- Numbers of astrocytes in the lumbar spinal cord and glial scarring were increased in TK mice at 5 weeks after injury.
- Our results point to differences in outcomes of astrocytic and cholinergic responses to CD11b-cell depletion compared to wild-type, which were, however, not reflected in the locomotor parameters analyzed at 5 weeks after spinal cord injury.