Anne Julia Céline Funke-Kaiser
Dr. med.

A Study on the Role of CD95L in the Innate and Adaptive Immune Response Following Spinal Cord Injury

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Doktormutter: Prof. Dr. med. Ana Martin-Villalba

We could show that CD95L is upregulated on peripheral neutrophils and monocytes in C57/BL6 mice following SCI. We established a FACS-staining protocol for CD95L surface expression on human peripheral blood leukocytes with the Nok2-clone showing high specificity on cells which were fixed with 4% PFA without losing sensitivity. Thereafter, we could detect elevated surface expression of CD95L on human neutrophils and lymphocytes. This indicates that an anti-CD95L therapy has to act in peripheral blood and not, as previously thought, locally in the injured spinal cord.

Neutrophils have received growing attention as a promising target to influence secondary damage of the injured spinal cord. This is mostly due to their early invasion to the lesion site 12-24 hours after injury and their potential to release neurotoxic and tissue degrading substances. Recently our group could show that pharmacological block of CD95L or exclusive deletion of CD95L in myeloid cells in $CD95L^{f/f;LysMcre}$ significantly reduced the number of neutrophils and macrophages invading the SC. This led to improved motor function and increased grey and white matter sparing. Therefore, we wanted to completely abrogate neutrophils. Application of the anti-granulocyte receptor-1 (Gr-1) mAb RB6-8C5 led to complete abrogation of peripheral and infiltrating neutrophils after SCI. We could show that complete depletion of neutrophils in C57/BL6 mice led to increased damage to neuronal tissue and consequently to worsened functional outcome compared to isotype-treated controls. This indicates that neutrophils and the inflammatory response which is mainly triggered and maintained by these cells within the first days after injury do not only do harm to the CNS. We assume that they have positive effects concerning tissue homeostasis, wound healing and regeneration. Mice with an exclusive deletion of CD95L in myeloid cells ($CD95L^{f/f;LysMcre}$ mice) show a reduced number of infiltrating neutrophils but improved functional outcome. Altogether, these data support the hypothesis that CD95L acts on the innate immune response to induce tissue damage and that the residual inflammation exerts a certain beneficial effect.

Being fundamental participants in primary host defence, we evaluated whether Ly6C^{high}, proinflammatory monocytes versus Ly6C^{low} monocytes are orchestrated in a specific way following spinal cord injury in C56BL/6N mice. The ratio of Ly-6C^{high}/Ly-6C^{low} shifted to the proinflammatory phenotype after spinal cord injury. In CD95L$^{f/f;LysMcre}$ this shift was less pronounced compared to their littermate controls. This is according to former results of our group that in CD95L$^{f/f;LysMcre}$ there can be found less inflammatory cytokines, less apoptosis, less degradation of white and grey matter and better functional outcome.

Concerning the adaptive immune response, we could not see any difference in the number of infiltrating T cells in the late phase 3 and 8 weeks after SCI between CD95L$^{f/f;LysMcre}$ and their littermate control. In conclusion, the recruitment of myeloid cells and the number of infiltrating cells in the early inflammatory response which are CD95L-dependent, do not influence the T cell response after several weeks. Alternatively, differences might only be detectable in transgenic mouse models with expanded T cell populations. Furthermore, we could show that in the initial phase after SCI there is a decrease of lymphocytes indicating an immune depression syndrome, as already described for other trauma models. The percentage of Tregs decreases as soon as one day after injury, finally reaching significantly increased levels one week after injury. This supports the idea of Tregs acting as anti-inflammatory, regenerative players in the healing phase after SCI. Surprisingly this “recovery” of regulatory T cells happens slightly earlier in CD95L$^{f/f;LysMcre}$ mice what supports the findings of the pro-inflammatory effect of CD95L on myeloid cells.