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The role of the CD95 system in Parkinson's Disease

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In this study, I examine the inflammatory response following death of dopaminergic neurons (DNs) and the role of CD95 therein in mouse models for Parkinsons's Disease (PD) (DT-model and subacute MPTP model).
Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. It is characterized by a slow and progressive degeneration of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc). Cardinal clinical manifestations are resting tremor, rigor, bradykinesia and postural instability. The MPTP model is the best characterized mouse model for PD and is based on the specific neurotoxicity to DNs of the MPTP metabolite MPP^+.
Neuroinflammation is increasingly recognized as a hallmark of Parkinson's disease. Activated CNS-resident microglia and infiltrating T-cells contribute to the degeneration of dopaminergic neurons (DNs). By contrast, the significance of peripheral innate immune cells for the progression of PD has not been examined thoroughly to date.
CD95 is a mediator of inflammation that has also been proposed as an apoptosis inducer in DNs, but previous studies using ubiquitous deletion of CD95 or CD95L in mouse models of PD have generated conflicting results. Whereas Fas null mice are more resistant to MPTP-induced death of DNs compared to WT controls, lpr and gld mice are more susceptible. The respective authors claim that activation of CD95 on DNs induces apoptosis or has neuroprotective effects, respectively. However, given global deficiency of CD95/CD95L in these mice, the effects can be hardly attributed uniquely to CD95 on DNs. In contrast, spinal cord neurons are not killed through direct CD95-mediated cell death after spinal cord injury in mice. Of note, the authors used mice with specific deletion of CD95L in all neurons ($CD95L^{fl/fl;\ Netin\text{-}Cre}$).
Thus, I sought to clarify the role of CD95 on DNs in PD using mice with exclusive deletion of CD95 ($CD95L^{fl/fl;\ DAT\text{-}Cre}$). I demonstrate that following MPTP intoxication mice with exclusive deletion on DNs neither are more susceptible, nor more resistant than control animals. In conclusion, DNs are not killed through direct CD95-mediated cell death.
As aforementioned, mice with global deficiency of CD95 (Fas null) have been described as more resistant to MPTP. However, if exclusive deficiency of CD95 on DNs does not reduce depletion of DNs, where does neuroprotection in mice with global deficiency of CD95 come from? In the acute MPTP model, T-cells infiltrating the SNpc contribute to degeneration of DNs. Depletion of $CD4^+$ T-cells makes mice more resistant to MPTP. Remarkably, microgliosis is ameliorated in these mice. In addition, specific deficiency in CD95L on myeloid cells results neuroprotective in spinal cord injured mice.
Inspired by these results I meant to examine the pathologic role of CD95L on myeloid cells in the MPTP PD model. I show that mice with specific deletion of CD95L ($CD95^{fl/fl;\ LysM\text{-}Cre}$) in the myeloid lineage (granulocytes, monocytes, microglia) are protected against MPTP induced neurodegeneration. Importantly, microgliosis is alleviated in these mice. In conclusion, CD95L on myeloid cell contributes to dopaminergic neurodegeneration in MPTP intoxicated mice.
Yet, CD95L of which myeloid population is to blame? Resident microglia or infiltrated peripheral myeloid cells? To date, infiltration of myeloid cells has not been examined appropriately in models of PD. Importantly, infiltrating monocytes or monocyte-derived

microglia, respectively, considerably contribute to disease progression in mouse models of EAE and ALS. In these mice accumulation of inflammatory monocytes can already be observed at preclinical stages. In addition, following spinal cord injury, infiltration of the spinal cord by myeloid cells has been found reduced in mice with specific deletion of CD95L in myeloid cells.

Thus, I was interested to find out if infiltration of monocytes plays a role in PD and if CD95L is involved in this process. In order to examine this, one group received intraperitoneal injections of APG112 (an anti-CD95L antibody, APOGENIX®) on the first and last day of MPTP intoxication. I did not observe extravasation of APG112 in brain slices of SNpc. Therefore I assume that APG112 only neutralized CD95L on blood leukocytes. I observed an accumulation of $CD115^{+}$ $Ly6C^{hi}$ inflammatory monocytes in MPTP treated mice that was 1,5-fold increased compared to Saline treated mice. Remarkably, as assessed by FACS, numbers of inflammatory monocytes are significantly reduced in mice that additionally received APG112. In addition, the same mice exhibit a significantly alleviated depletion of DNs in the SNpc. Importantly, immunofluorescent staining against P2Y12 (exclusively expressed on resident but not on monocyte-derived microglia) and CD11b revealed that infiltrating monocytes ($CD11b^{+}$ $P2Y_{12}^{-}$) contribute to microgliosis in the SNpc. Of note, accumulation of monocyte-derived microglia is significantly reduced in mice additionally treated with APG112.

In conclusion, CD95L on circulating myeloid cells mediates their accumulation in the blood and in the SNpc in an autocrine manner and thus contributes to neurodegeneration in MPTP intoxicated animals. However, it remains to be eludicated if the neuroprotective effect by anti-CD95L is predominantly based on inhibition of the peripheral innate or adaptive cellular immunity or even endothelial cells.

In summary, this study highlights the pathological significance of the peripheral innate immune response in the progression of neurodegeneration and identifies the CD95/CD95L system as crucial trigger of this inflammatory response in PD. Thus, I propose systemic neutralization of CD95L as a potential therapy in PD.