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LETTER TO THE EDITOR

CD95/Fas mediates cognitive improvement after traumatic brain injury

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CD95 (APO-1/Fas)/CD95L (APO-1L/FasL/CD178) is a receptor/cytokine pair of the tumor necrosis factor/nerve growth factor (TNF/NGF) superfamily. Similar to other receptors of this family, activation of CD95/Fas results in the formation of a death-inducing signaling complex (DISC) and subsequent activation of the apoptotic cascade [1]. There is increasing evidence that CD95/Fas also signals survival, proliferation, and migration in CD95/Fas-expressing cells that are resistant to CD95L/FasL-induced cell death [2, 3]. This non-apoptotic signaling of CD95/Fas is also referred to as "reverse signaling" [3]. In contrast to the apoptotic signaling cascade, the molecular mechanisms resulting in the activation of non-apoptotic pathways are still vague [3].

The physiological role of CD95/Fas expression in the central nervous system is unknown. Both, CD95/Fas and CD95L/FasL have been detected in the brain [4-6], but their spatial and temporal expression patterns change during development [5, 7]. Mice with defective CD95/Fas receptor (lpr-mice) or ligand (gld-mice) are protected in various animal models of acute neuronal damage, e.g. after ischemic stroke or spinal trauma [8, 9]. Zuliani and coworkers provided first evidence that CD95/Fas-mediated "reverse signaling" might mediate neuroregeneration in the CNS [10]. They could show that CD95/Fas did not induce apoptosis in cortical and hippocampal neurons but rather controlled branching of these neurons *in vitro*. In addition, the authors found a significant difference in the number of dendritic branches in mice deficient in CD95/Fas and CD95L/FasL, suggesting an involvement of this mechanism during embryonic development. It is unknown whether CD95/Fas-controlled neuronal branching might be relevant in neurological diseases [10]. Because neuronal branching significantly contributes to neuroplasticity and therefore neurological recovery after acute neuronal damage, we speculated that CD95/Fas might be involved in the regeneration after traumatic brain injury (TBI). To test this hypothesis, we investigated the acute and chronic responses of CD95/Fas-deficient lpr-mice and wildtype control animals after controlled cortical impact (CCI).

CCI is a well-established animal model for TBI. It was performed using MRL-lpr mice and wildtype control animals with the same genetic background (composite genomic background: LG (75%), AKR/J (12.6%), C3H (12.1%), C57BL/6 (0.3%), Harlan UK Limited, Great Britain) as described previously (CCI: 2 mm flat-tip impounder; pressure: 25 kPa; depth: 1 mm, impact duration: 100 ms) [11]. Because MRL-lpr-mice develop systemic vasculitis at the age of 12 weeks [12], we used 6-8-week-old animals for the experiments. No signs of vasculitis were seen on hematoxylin/eosin-stained slides. To rule out a subconscious investigator bias, CCI, histological evaluation, and behavioral tests were performed "blinded".

The CCI lesion was placed over the motor cortex causing direct damage of motor cortex and indirect, time-dependent cell death of hippocampal neurons (Figure 1A). We used the beam walking test [13] to determine locomotor deficits and the Morris water maze test [14] to monitor the hippocampal function. In line with previous results, lpr-mice performed significantly better in both tests during the first 72 h after

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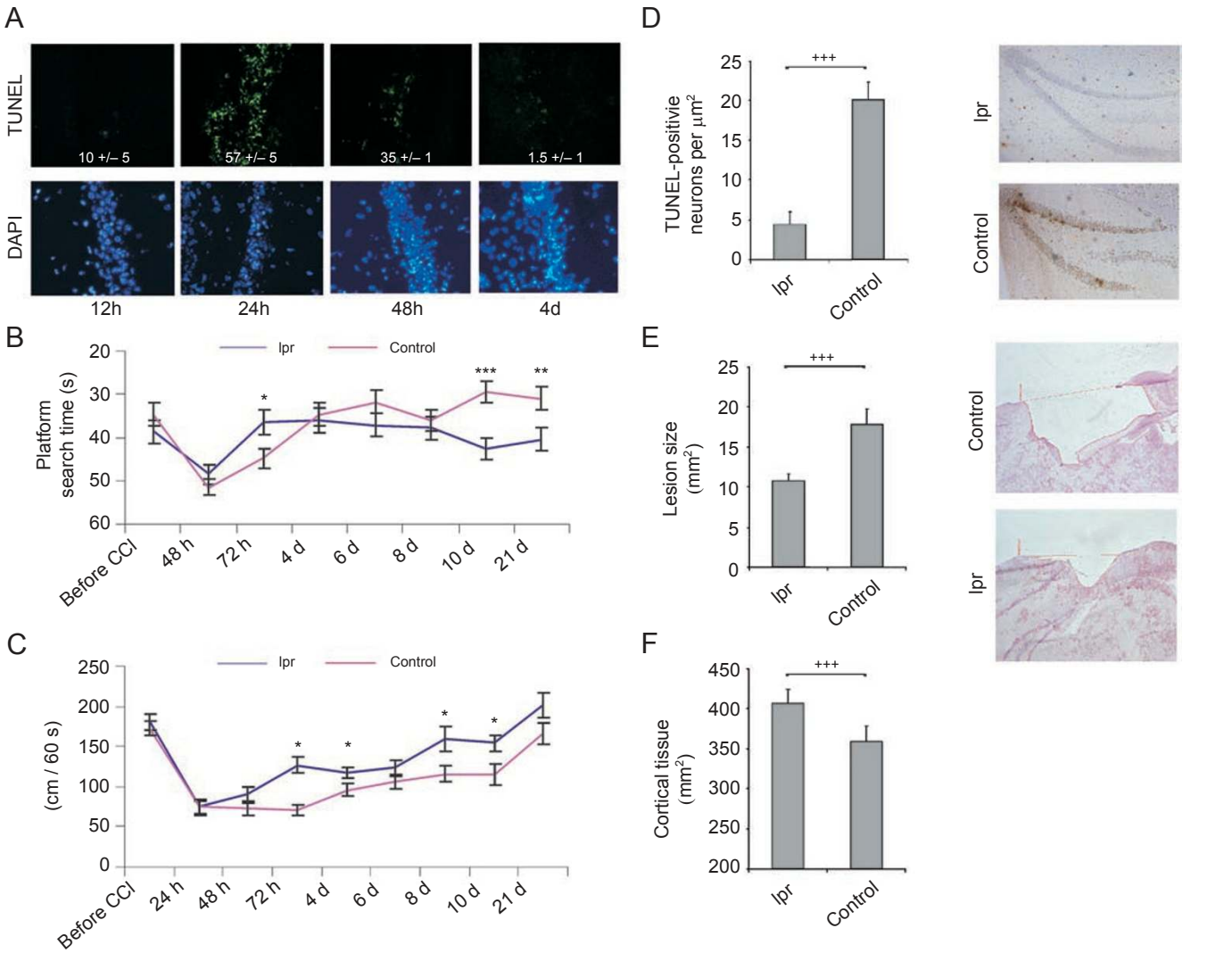

Figure 1 CD95/Fas mediates cognitive improvement after CCI. **(A)** Time course of indirectly damaged hippocampal neurons after CCI. Apoptotic neurons in the dentate gyrus of the hippocampus in wildtype animals were detected using the Apo-direct Kit (Chemicon, München, Germany) for TUNEL (Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End-Labeling) staining. The average number of apoptotic neurons in the dentate gyrus per slide at the time points indicated is given (n = 4 animals). **(B)** Morris water maze test: Mice (lpr n = 8, control n = 8) learned to find the position of an invisible platform in a black water tank (diameter: 1.05 m with white signs for orientation) on 3 consecutive days before CCI. After CCI, the mice had to find the platform within 60 s, where they were allowed to sit for 10 s (5 cycles per timepoint). The platform position remained unchanged during the entire experiment; the mouse was placed randomly in the tank. If the mouse did not find the platform within 60 s, it was placed on the platform for 10 s to enable it to memorize its position. The average latency until the mice found the platform is shown. In all, 6 trials per time point and animal were performed. We did not conduct the Morris water maze test 24 h after CCI to avoid wound infections. **(C)** Beam walking test: The mouse was placed on an aluminum beam (diameter: 0.6 cm, length: 60 cm), from which it had to reach a large platform. The distance that the mouse covered within 60 s was determined. The test was repeated 4 times per time point. If a mouse fell down the beam or did not arrive within 60 s at the arrival platform, the distance until it fell down was measured. The mice learned to walk on the beam 6 times on 3 consecutive days before CCI. **(D)** At 24 h after CCI, lpr-mice had significantly fewer TUNEL-positive neurons in the dentate gyrus of the hippocampus compared with control animals. The number of TUNEL-positive neurons per μm^2 is given. A total of 8 sections per animal (lpr n = 5, control n = 4) were analyzed. Representative pictures are shown. **(E, F)** The lesion volume and the size of the remaining brain tissue were determined after completion of the neurological testing 22 days after CCI (lpr n = 8, control n = 8). The average sizes of the lesion **(E)** and the remaining cortical tissue **(F)** are shown. In all, 5 sections per animal were assessed, representative lesions are shown (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, one-way ANOVA; +++ $p < 0.001$, Student's t-test; mean and SEM are given).

the trauma (Figure 1B and 1C). The reduced neurological deficits were paralleled by a significantly reduced number of TUNEL-positive hippocampal neurons in lpr- as compared with wildtype mice 24 h after CCI (Figure 1D).

We further tested the mice until 21 days after the trauma. As compared with lpr-mice, wildtype mice had enduring and more pronounced locomotor deficits (Figure 1C) due to the direct damage of the motor cortex and subsequent larger lesions (Figure 1E) with less remaining brain tissue (Figure 1F). Notably, wildtype mice that had performed worse in the Morris water maze test directly after CCI improved their spatial memory function faster and better as compared with lpr-mice (Figure 1B).

The Morris water maze test determines the spatial memory function and the performance correlates with the integrity of hippocampal neurons that strongly express CD95/Fas [5, 14]. The impaired improvement of the spatial memory function of lpr-mice after CCI therefore implies a functional relevance of the "death receptor" CD95/Fas expression on hippocampal neurons. Because neuronal branching is a major mediator for regeneration and subsequent functional recovery, it appears likely that CD95/Fas-mediated neuronal branching is involved in the cognitive improvement after CCI [10]. Our results therefore complement the *in vitro* data published by Zuliani *et al*. and suggest a functional relevance of CD95/Fas-mediated neuronal branching in this paradigmatic animal model.

CD95/Fas has a two-facet role in the pathogenesis of TBI. It is detrimental directly after the trauma and seems to become a mediator of regeneration by mediating neuronal branching in the later course of the disease. These conflicting functions are most likely mediated by regulatory proteins (e.g. LFG or c-FLIP) that are expressed in the hippocampus [6, 15] and regulated after TBI [15]. Therefore, targeting these proteins might be an excellent therapeutic approach for TBI aiming to inhibit apoptotic neuronal death initially and to promote neuronal recovery later on.

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