Direct evidence that activating the interleukin 1 type I receptor enhances ischemic brain damage

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INTRODUCTION: The inflammatory response is proposed to contribute to progressive neurodegeneration following acute CNS insults and chronic degenerative disorders such as Alzheimer's and Parkinson's disease. Upon insult, proinflammatory cytokines are rapidly induced and secreted, leading to a cascade of cytotoxic effects known to exacerbate the damage subsequent to injury (1). Interleukin-1 (IL-1) is known to enhance the extent of damage subsequent to ischemia, however, the receptor(s) that mediates its effects remains unclear (2). To establish whether the IL-1 type 1 Receptor (IL-1R1) is necessary for IL-1 exacerbated brain damage, we employed a modified Levine model of hypoxia-ischemia (H/I) on adult mice (2). We used MRI to determine the extent of hyperintense T2 signal prior to and after IL-1β injections in this model. We tested the following hypothesis: if the activation of IL-1 Receptor Type1 (IL-1R1) is responsible for enhancement of H/I damage in response to IL-1 cytokine, then the mouse knockout for IL-1R1 will be protected following addition of IL-1, after H/I.

METHODS: Male IL-1R1 null and wild-type (WT) mice, 2 to 3 months old, of a C57BL/6 background were used. The animals were anesthetized with isoflurane (4% induction, 1.5% maintenance in air) and the right carotid artery was double ligated. To induce hypoxia, each animal was exposed to a gas mixture of 8% O_2/balance N_2 for 20 min. MR imaging was performed on a 3.0 T MRI spectrometer (Medspec S300, Bruker Instruments, Ettlingen, Germany). Wild type and IL-1R1 null mice were imaged at 24 h post H/I. At 48h following H/I mice (6 IL-1R1 null and 10 WT) were injected with 5 ng/ml of IL-1b in Hank's balanced salt solution (HBSS) into the contralateral lateral ventricle, and control animals were injected with HBSS only. At 72 h, two animals per group were sacrificed for histology. Imaging was repeated at 7 days following H/I. Each mouse was imaged using a 2.5 cm slotted tube resonator. The H/I injury prior and after IL-1β injections was assessed from ten continuous coronal slices 0.5 mm thick, acquired with TR/TE=3000/69.4 ms, field of view 2 x 2 cm^2, matrix 256 x 256, and 8 averages in 6 min, using a RARE imaging sequence. To quantify T2 changes over a 7-days period, each mouse was imaged with a multi echo spin echo sequence in addition to RARE, at 24 h and 7 days. The spin echo sequence had TR/TE=3000/10.13-151.95 ms, 128 X 128 matrix size and N=15 echoes, 2 averages in 13 min, with the same slice thickness and position as RARE imaging. Slices were segmented by setting an intensity threshold using the CCHIPS/IDL software (3). Infarct volumes were corrected for brain edema as described previously (4), and calculated as percent of the hemisphere. T2 maps were calculated on a pixel-by-pixel basis from the multi echo images. Immunohistochemisty using microglia specific marker (Iba-1) and astrocytic marker (GFAP) was performed at 48 h, on cryostat sections, and fluorescent secondary antibodies were used for visualization.

RESULTS: At 7 days following H/I, the volume of hyperintense T2-signal in WT animals injected with IL-1β was *increased* 8% compared to the initial damage volume at 24 h (Fig. 1). By contrast, the volume of hyperintense T2-signal in IL-1R1 null animals injected with IL-1β was 5% *reduced* compared to the initial damage volume at 24 h (Fig. 1). The difference in preserved brain tissue between IL-1R1 null and WT animals injected with IL-1β was statistically significant ($p < 0.05$). At the cellular level at 72 h, fewer amoeboid microglia/macrophages and fewer GFAP-positive cells were present in the neocortex of IL-1R1 null mice compared to WT mice following the IL-1β injections. There was no noticeable difference in the numbers of reactive microglia and astrocytes in the striatum.

CONCLUSIONS: The presented results demonstrate that the absence of signaling through the IL-1R1 prevented increase in T2 values as a consequence of IL-1β injections and H/I. These findings strongly indicate that progressive neurodegeneration in response to IL-1β cytokine requires intact IL-1R1 receptor signaling. Therapeutic modalities aimed toward suppression of IL-1R1 signaling may hold promise to preserve neocortical function after stroke.

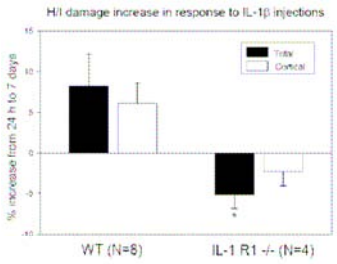

Figure 1. Differences in the H/I injury between 7 days and 24 h. There was an increase in the total and cortical volume of the hyperintense T2-weighted signal in WT animals from 24 h to 7days, as a consequence of IL-1β injections. In contrast IL-1R1 null animals had significant reduction of injury volume at 7 days compared to WT.

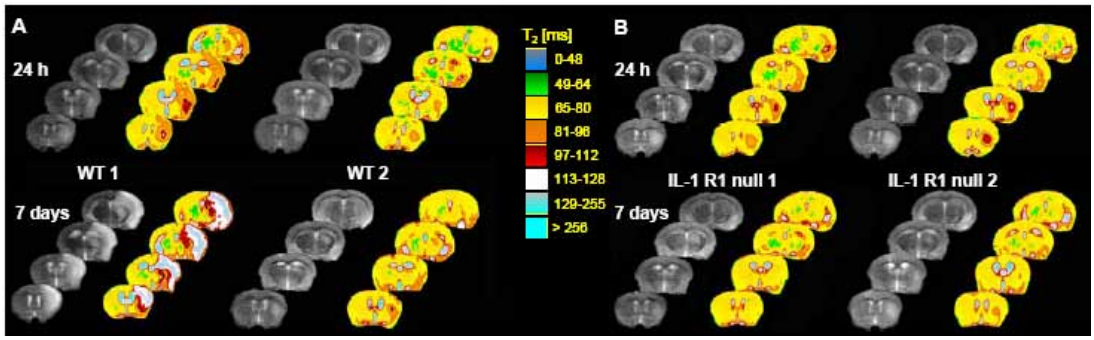

Figure 2. Representative MRI of two different WT animals (A) at 24 h and 7 days, injected with IL-1β at 48h, and two different IL-1R1 null animals (B). Notice the increase in T2-values in WT mice between 24 h and 7 days, and decrease in T2-values in IL-1R1 null animals. Color scale represents different T2 values.

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