Inflammasome activation and reduced sTREM2 release in LPS-stimulated organotypic brain slices

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BACKGROUND

Immune activation in the CNS and production of neurotoxic mediators are linked to various neurodegenerative diseases, especially to Alzheimer's disease (AD). On the one hand, the finding of TREM2 variants as genetic risk factor for AD and on the other hand, the current focus of research on the inflammasome activation are giving evidence for the importance of glial cells in AD. Further characterization of glial cells, ideally in co-culture with neurons, during inflammatory processes are necessary to better understand the disease. Also screening of anti-inflammatory drug candidates in an *in vitro* system maintaining the interplay of different cell types is highly relevant to provide results of translational value.
To assess whether organotypic slices are a suitable tool to investigate inflammasome activation and involvement of TREM2 we tested several lipopolysaccharide (LPS) stimulation paradigms in this system.

MATERIALS and METHODS

To study neuroinflammation in an intact neuronal system, organotypic hippocampal slices were prepared from early postnatal wild type mouse pups. To stimulate inflammation, slices were incubated with LPS at numerous concentrations and cytokine as well as sTREM2 release into the supernatant was measured by multiplex Mesoscale Discovery (MSD) immunosorbent assay at several time points. NLRP3 expression was examined with protein simple WES technology. Dexamethasone or MCC950 served as reference items to counteract the inflammatory response.

RESULTS

LPS stimulation at numerous concentrations and durations differently changed cytokine and sTREM2 release into the supernatant as well as NLRP3 expression within the slices. Reference items like Dexamethasone or MCC950 were able to downregulate the inflammatory response to various degrees.

SUMMARY and CONCLUSION

Organotypic brain slices are a perfect tool to study neuroinflammatory pathways *ex vivo*. Disease-relevant pathways are activated by LPS stimulation and this process can be reversed by some reference compounds. By maintaining the three-dimensional structure and interplay of different cell types of the postnatal brain, this system closely resembles the *in vivo* situation while offering several advantages for early screenings, like sampling of supernatant at various time points and higher through-put.

RESULTS

sTREM2 Protein Release

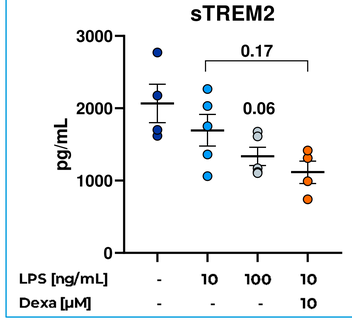

Figure 1: sTREM2 release of hippocampal brain slices after 24 h LPS incubation. Organotypic hippocampal brain slices were incubated with 10 ng/mL or 100 ng/mL LPS as well as with 10 ng/mL LPS in combination with 10 µM Dexamethasone (Dexa) for 24 h, followed by detection of TREM2 in the supernatant by MSD. One-Way ANOVA with Bonferroni's multiple comparisons test vs. control. Mean ± SEM; n = 4-5.

RESULTS

Cytokine Release

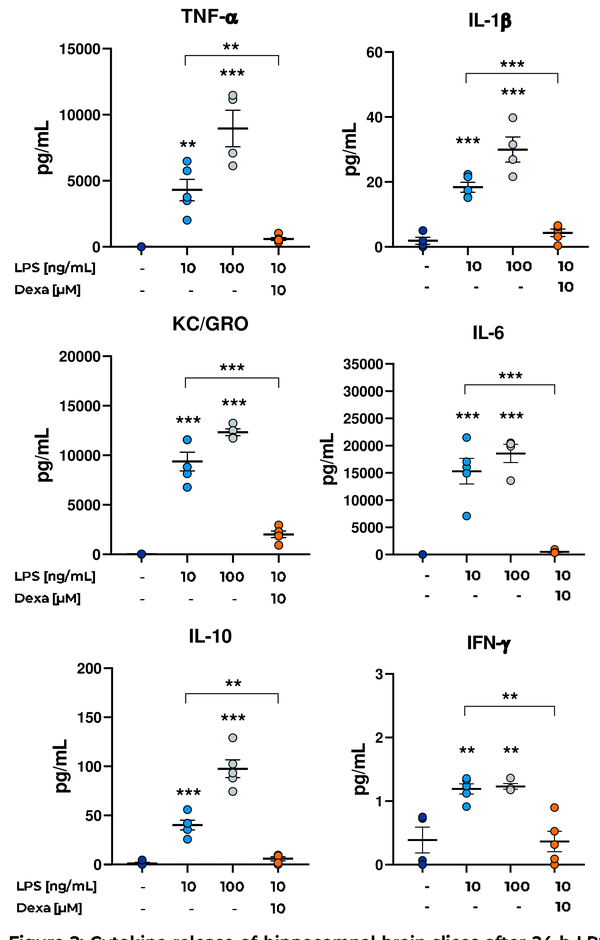

Figure 2: Cytokine release of hippocampal brain slices after 24 h LPS incubation. Organotypic hippocampal brain slices were incubated with 10 ng/mL or 100 ng/mL LPS as well as with 10 ng/mL LPS in combination with 10 µM Dexamethasone (Dexa) for 24 h, followed by detection of cytokine release in the supernatant by MSD. One-Way ANOVA with Bonferroni's multiple comparisons test vs. control. Mean ± SEM; n = 4-5. **p<0.01., ***p<0.001.

RESULTS

Cytokine Release

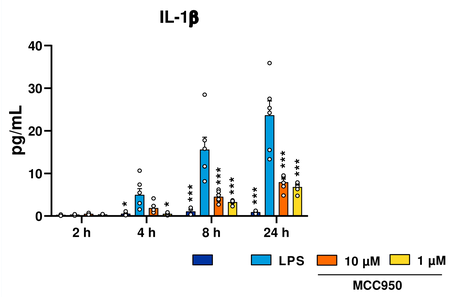

Figure 3: IL-1β and TNF-α release of hippocampal brain slices after 2 h, 4 h, 8 h and 24 h LPS incubation. Organotypic hippocampal brain slices were incubated with 100 ng/mL LPS in combination with 10 µM or 1 µM MCC950, followed by detection of IL-1β and TNF-α levels in the supernatant by MSD. One-Way ANOVA with Bonferroni's multiple comparisons test vs. LPS. Mean + SEM; n = 6. *p<0.05, ***p<0.001.

NLRP3 Protein Expression

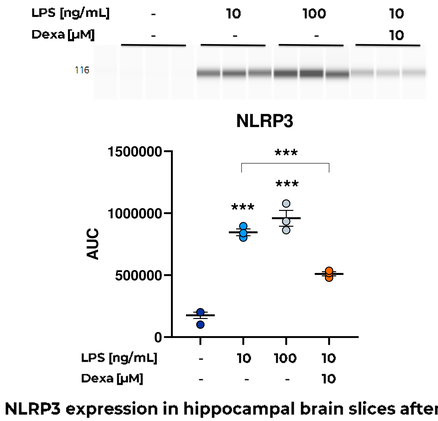

Figure 4: NLRP3 expression in hippocampal brain slices after 24 h LPS incubation. Organotypic hippocampal brain slices were incubated with 10 ng/mL or 100 ng/mL LPS as well as with 10 ng/mL LPS in combination with 10 µM Dexamethasone (Dexa) for 24 h, followed by detection of NLRP3 protein expression in RIPA lysates by WES. One-Way ANOVA with Bonferroni's multiple comparisons test vs. control. Mean ± SEM; n = 4. ***p<0.001.

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