Comparison of two different *in vitro* models of Neuroinflammation: Cytokine release and Inflammasome activation in organotypic brain slices and BV-2 cells

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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.

In the current study, two different LPS-stimulated *in vitro* systems of neuroinflammation were assessed in terms of cytokine release, sTREM2 levels and expression of the inflammasome component NLRP3. The response to LPS treatment of organotypic brain slices, a mixed-model with intact cell-cell interactions, was compared to the response of the immortalized murine microglial cell line BV-2.

MATERIALS and METHODS

Organotypic hippocampal brain slices were prepared from early postnatal C57BL/6 mouse pups. To stimulate inflammation, slices were incubated with LPS and cytokine as well as sTREM2 release into the supernatant was measured by Mesoscale Discovery (MSD) immunosorbent assay. NLRP3 expression was examined with protein simple WES technology. Dexamethasone and MCC950 served as reference items to counteract the inflammatory response. A comparable treatment paradigm was used for BV-2 cells.

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RESULTS

LPS stimulation differently changed cytokine and sTREM2 release into the supernatant as well as NLRP3 expression within organotypic brain slices and BV-2 cells. The reference items were able to downregulate the inflammatory response to various degrees.

RESULTS

Organotypic brain slices

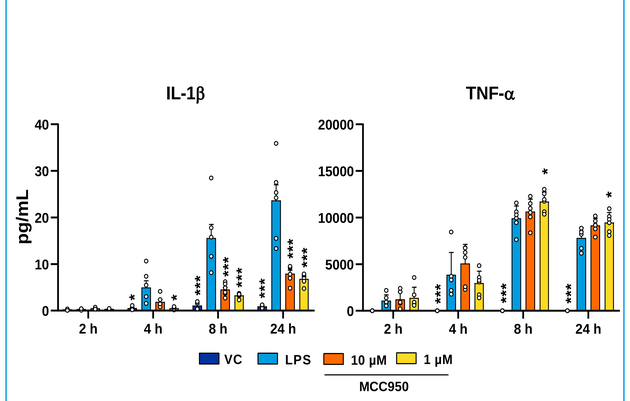

Figure 1: IL-1β and TNF-α release of hippocampal brain slices after 2 h, 4 h, 8 h and 24 h LPS incubation. Organotypic hippocampal brain slices were incubated with 100 ng/mL LPS in combination with 10 µM or 1 µM MCC950, followed by detection of IL-1β and TNF-α levels in the supernatant by MSD. One-Way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

SUMMARY and CONCLUSION

Disease-relevant inflammatory pathways are activated by LPS stimulation in both systems and this process can be reversed by reference compounds. While BV-2 cells can serve as high-through-put screening tool, organotypic brain slices closely resemble the *in vivo* situation offering several advantages for early screenings, like maintenance of the interplay of different cell types in the postnatal brain.

RESULTS

Organotypic brain slices

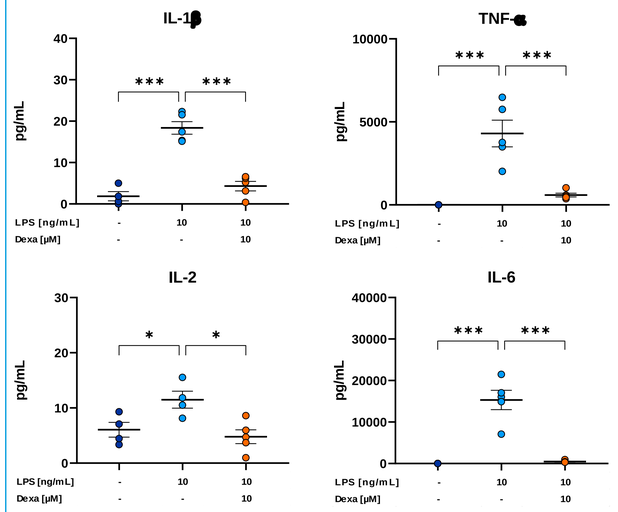

Figure 2: Cytokine release of hippocampal brain slices after 24 h LPS incubation. Organotypic hippocampal brain slices were incubated with 10 ng/mL LPS as well as with 10 ng/mL LPS in combination with 10 µM Dexamethasone (Dexa) for 24 h, followed by detection of cytokine release in the supernatant by MSD. One-Way ANOVA with Dunnett's multiple comparisons test vs. LPS. Mean ± SEM; n = 4-5. *p<0.05, ***p<0.001.

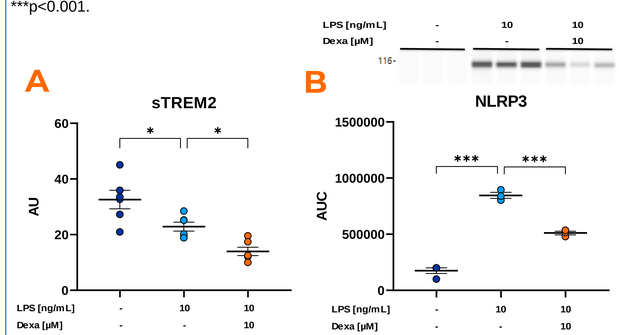

Figure 3: sTREM2 release and NLRP3 expression of hippocampal brain slices after 24 h LPS incubation. Organotypic hippocampal brain slices were incubated with 10 ng/mL LPS as well as with 10 ng/mL LPS in combination with 10 µM Dexamethasone (Dexa) for 24 h, followed by detection of (A) TREM2 in the supernatant by MSD and (B) NLRP3 protein expression in RIPA lysates by WES. One-Way ANOVA with Dunnett's multiple comparisons test vs. LPS. Mean ± SEM; n = 4-6. *p<0.05, ***p<0.001.

RESULTS

BV-2 cells

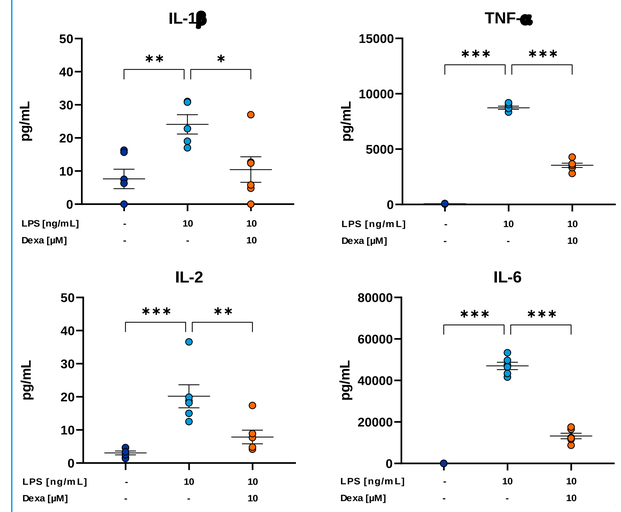

Figure 4: Cytokine release of murine microglial BV-2 cells after 24 h LPS incubation. BV-2 cells were incubated with 10 ng/mL LPS as well as with 10 ng/mL LPS in combination with 10 µM Dexamethasone (Dexa) for 24 h, followed by detection of cytokine release in the supernatant by MSD. One-Way ANOVA with Dunnett's multiple comparisons test vs. LPS. Mean ± SEM; n = 4-5. *p<0.05, **p<0.01, ***p<0.001.

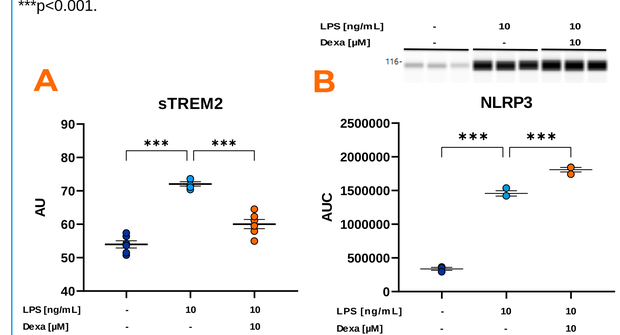

Figure 5: sTREM2 release and NLRP3 expression of murine microglial BV-2 cells after 24 h LPS incubation. BV-2 cells were incubated with 10 ng/mL LPS as well as with 10 ng/mL LPS in combination with 10 µM Dexamethasone (Dexa) for 24 h, followed by detection of (A) TREM2 in the supernatant by MSD and (B) NLRP3 protein expression in RIPA lysates by WES. One-Way ANOVA with Dunnett's multiple comparisons test vs. LPS. Mean ± SEM; n = 4-6. ***p<0.001.