Comparison of two different in vitro models of Neuroinflammation: Cytokine release and Inflammammasome 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 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 use.

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 TREM2 release into the supernatant was measured by Mesoscale Discovery (MSD) immunosorbant 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.

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<0.001.

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 - 6. **p<0.01, ***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 extracts by WES. One-Way ANOVA with Dunnett’s multiple comparisons test vs. LPS; Mean ± SEM; n = 4 - 6. **p<0.01, ***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-throughput 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.

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