

SMALL MOLECULE ANTI- INFLAMMATORY COATING AND HYDROCEPHALUS SHUNT OBSTRUCTION

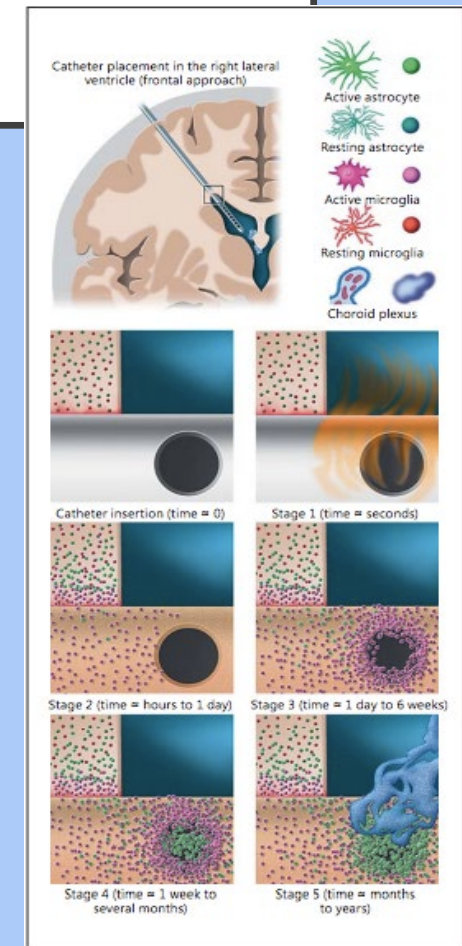
Neurological Surgery Summer Program, 2017

Chris Keim

University of Vermont 18'

SHUNT OBSTRUCTION

- Hydrocephalus is the accumulation of CSF in the brain.
- 30-40% of shunts placed for the treatment of hydrocephalus require replacement at 1 year post-op.
 - 50% at 2 years post-op
 - >90% at 10 years post-op
- 50% of pediatric shunt failures are caused by non-infectious catheter obstruction.



(Hanak et al., 2017)

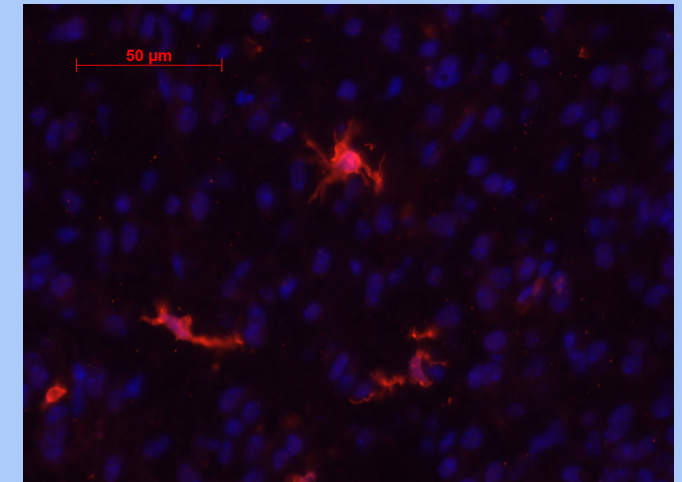
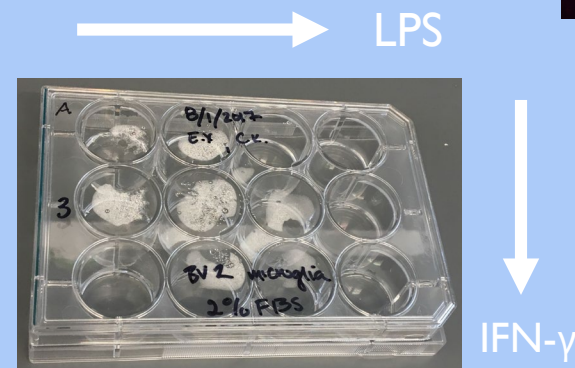
PREVIOUS WORK

- Anti-inflammatory triazole scaffold small molecule coatings (E9 and RZA15) developed at MIT (Langer & Anderson laboratory).
- Intra-ventricular catheters coated with either E9 or RZA15 showed significantly reduced immune response to catheter in rabbits.
- Microglia are the first responding cells to the shunt, and thus the first 'layer' of obstruction – how might the coating interact with microglia?



BV2 MICROGLIA

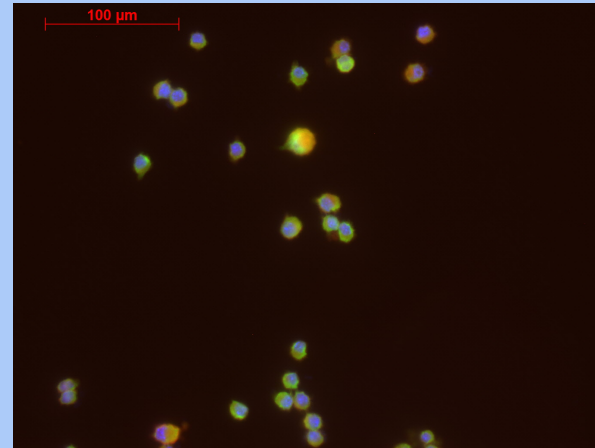
- Immortalized cell line, derived from murine primary microglia.
 - 90% similar gene expression with primary microglia in response to LPS.
- BV2 microglia placed into 12 well plates containing differing concentrations of LPS and IFN- γ .
 - Well 1 'lowest stimulation' \rightarrow Well 12 'highest stimulation'
- Immunohistochemistry and the Griess reaction.



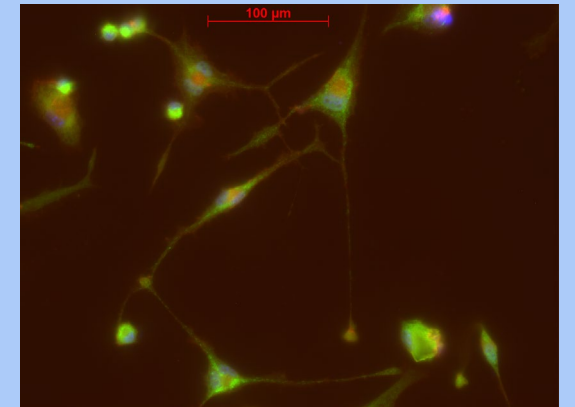
In vivo α -Iba1 immunofluorescence of microglia, 40x.

BV2 MICROGLIA IMMUNOHISTOCHEMISTRY

- Microglia consistently displayed 'amoeboid' morphology in low LPS and IFN- γ conditions.
- As the respective doses of LPS and IFN- γ increased, microglia increasingly displayed 'rod' phenotype.



Well 1 'low stimulation.' α -NF κ B (green) and α -Rhodamine-Phalloidin (red) in vitro, 20x.

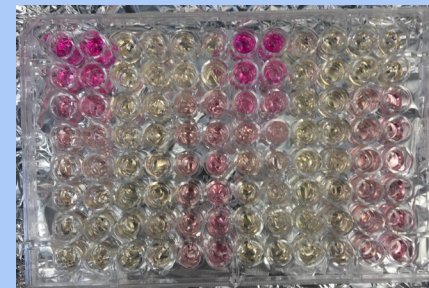
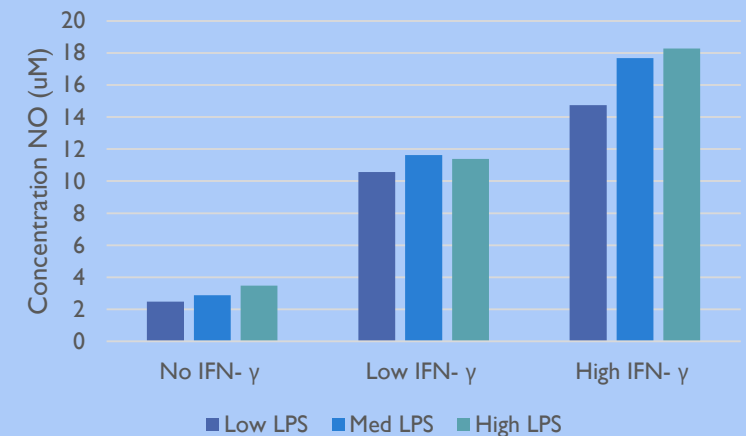


Well 12 'high stimulation.' α -NF κ B (green) and α -Rhodamine-Phalloidin (red) in vitro, 20x

GRIESS REACTION

- Measures nitric oxide (NO) formation, biomarker of inflammation.
- 96 well plates with media from BV2 microglia culture (varied LPS and IFN- γ concentrations) underwent Griess reaction, read using a plate reader measuring absorbance.
- Nitric oxide (NO) concentrations increased as LPS and IFN- γ concentrations increased.

NO concentration as a fxn of LPS and IFN- γ stimulation.





FUTURE DIRECTIONS

- Repeatable experimental culture and stimulation conditions for BV2 microglia and immunohistochemical assays.
- It is possible to determine a mechanism for RZA15's anti-inflammatory action, using molecules that activate specific immune signaling pathways in microglia.

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The logo for UW Medicine Neurological Surgery is a dark purple rectangular box with white text. The words "UW Medicine" are in a serif font, and "NEUROLOGICAL SURGERY" is in a sans-serif font, separated by two horizontal white lines.



THANK YOU!

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Translational Neuroscience and Neurological Surgery*