Endothelial targets of Blood-Brain Barrier Breakdown in bacterial Meningitis

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Background and preliminary data:
Bacterial meningitis is a severe infectious disease most commonly caused by Streptococcus pneumoniae, Neisseria meningitidis and Hemophilus influenzae. Despite receding incidence due to advanced vaccine-development and roll-out, infections still lead to high rates of mortality and lasting disability worldwide (1). Blood-Brain Barrier (BBB) Breakdown is a major pathological hallmark involved in meningitis and leads to increased influx of bacteria, immune cells and vasogenic edema and subsequently poses the risk of brain herniation with fatal consequences (1). As such, the discovery of targets that limit BBB breakdown could offer great benefit as adjunctive therapy besides antimicrobial agents. In our laboratory, we have identified several key mechanisms responsible for the breakdown of the blood-brain barrier (2, 3). Building on these findings, we now aim to elucidate how these processes drive blood-brain barrier disruption in the context of meningitis. Understanding the precise molecular and cellular events involved will inform novel therapeutic approaches to mitigate or prevent the devastating neurological outcomes associated with this condition.
G-Protein coupled receptors (GPCR) are the largest mammalian receptor family. They are abundantly expressed on brain endothelial cells and have been shown to influence BBB integrity (4). Furthermore, their therapeutic potential is underlined by the fact that roughly 30% of marketed drugs target a GPCR. GPCRs couple to specific and sometimes multiple subunits of G-Proteins (Gαs, Gαi, Gαq/11, and Gα12/13) that mediate their intracellular effects. To study their effects on the BBB we have developed a mouse model that allows the activation of Gαq/11 specifically in brain endothelial cells using Designer Receptors Exclusively Activated by Designer Drugs (DREADD). DREADD are mutated GPCRs that are specific for a G-Protein subunit and can only be activated by manufactured ligands (5). Expressing the Gαq/11-DREADD under a brain endothelial specific promotor (Slco1c1-CreER^{T2}) allows us to study its role in a cell and time-specific manner. Preliminary results have shown that activation of endothelial Gαq/11 leads to a rapid BBB opening and lethality after 90 minutes, similar to the clinical presentation seen in patients with pneumococcal meningitis. We aim to build upon this novel finding and further investigate the mechanisms of endothelial Gαq-dependent BBB breakdown using our transgenic mouse line, establish potential therapeutic targets and test these in a murine model of bacterial meningitis.

Hypothesis:
We hypothesize that Gq-dependent signaling pathways in endothelial cells play an important role in BBB breakdown following bacterial meningitis.

Aims and Work Programme:
1. We will use the Slco1c1-CreER^{T2}x Gαq/11-DREADD mouse to characterize endothelial Gαq/11-dependent BBB breakdown
2. Identifying Gαq/11-dependent targets in a murine model of bacterial meningitis
3. Integrating the results from Aims 1 and 2 to identify novel endothelial targets for modulation of BBB breakdown and test these in our meningitis model

Aim #1: Characterizing Slco1c1-CreER^{T2}x Gαq/11-DREADD mouse
We have established the Slco1c1-CreER^{T2}x Gαq/11-DREADD mouse line in our lab allowing us to specifically activate Gaq-signaling in a brain endothelial- and time-specific manner. Preliminary results have shown that activation of these DREADD lead to a rapid neurological deterioration and BBB breakdown as assessed by Evans Blue dye extravasation, indicating a prominent and so far poorly understood mechanism of BBB damage. To characterize this observation, we will use MRI including Perfusion imaging to assess differences in cerebral blood flow and edema formation. Furthermore, histological assessment and Western Blotting

of endothelial junction proteins will be performed to assess the parenchymal injury. We have also established a brain endothelial cell line (bEND.3) stably transfected with the Gαq/11-DREADD construct to further assess barrier properties via electrical cell-substrate impedance sensing (ECIS) and cytochemistry. Performing RNA Sequencing of these activated endothelial cells at different time-points will help us identify downstream targets that mediate this BBB breakdown. To validate these results in vivo, we will use an established protocol to isolate endothelial nuclei for single-nucleus RNA-sequencing.

Aim #2: Identifying Gαq/11-dependent targets in a murine model of bacterial meningitis

We will establish a murine model of streptococcal meningitis as previously described (1). In brief, meningitis is induced by transcutaneous injection of 15 µl 10^7 colony forming units (cfu)/ml of *Streptococcus pneumoniae* (*S. pneumoniae*) type 3 into the cisterna magna. Performing single-nucleus RNA-sequencing of endothelial cells and correlating this with the sequencing results of our endothelial cells from Aim #1 will allow us to uncover preserved Gαq/11-dependent targets that are upregulated in experimental meningitis.

Aim #3: Isolating new therapeutical targets to limit BBB breakdown in bacterial meningitis

Building upon the potential endothelial candidate genes identified in Aim #1 and #2 we will first validate therapeutic targets in vitro using our Gq-DREADD endothelial cell line and ECIS measurements as a model of endothelial barrier breakdown. In a second step, promising targets will be tested in our murine model of bacterial meningitis. Outcomes will include clinical score, edema formation, CSF pleocytosis and ICP-measurements.

In conclusion, we believe that Gαq/11-dependent signaling in brain endothelial cells is a vital mediator of BBB breakdown. A better understanding of this process and its role in bacterial meningitis will help identify valuable therapeutic targets to limit the consequences of excessive brain edema as adjunctive therapy to antimicrobial treatment.

Project-related publications: (max. 5)

1. Teske, N. C., Dyckhoff-Shen, S., Beckenbauer, P., Bewersdorf, J. P., Engelen-Lee, J.-Y., Hammerschmidt, S., Kälin, R. E., Pfister, H.-W., Brouwer, M. C., Klein, M., Glass, R., van de Beek, D., and Koedel, U. (2023) Pericytes are protective in experimental pneumococcal meningitis through regulating leukocyte infiltration and blood–brain barrier function. *Journal of Neuroinflammation* **20**, 267
2. Ludewig, P., Sedlacik, J., Gelderblom, M., Bernreuther, C., Korkusuz, Y., Wagener, C., Gerloff, C., Fiehler, J., Magnus, T., and Horst, A. K. (2013) Carcinoembryonic antigen-related cell adhesion molecule 1 inhibits MMP-9-mediated blood-brain-barrier breakdown in a mouse model for ischemic stroke. *Circ Res* **113**, 1013-1022
3. Winneberger, J., Schols, S., Lessmann, K., Randez-Garbayo, J., Bauer, A. T., Mohamud Yusuf, A., Hermann, D. M., Gunzer, M., Schneider, S. W., Fiehler, J., Gerloff, C., Gelderblom, M., Ludewig, P., and Magnus, T. (2021) Platelet endothelial cell adhesion molecule-1 is a gatekeeper of neutrophil transendothelial migration in ischemic stroke. *Brain Behav Immun* **93**, 277-287
4. B S, G., B S, N., K, R., C S, M., and R, S. (2024) Unlocking the therapeutic capabilities of GPCR in the treatment of ischemic stroke: A translational literature. *Medicine in Drug Discovery* **24**, 100197
5. Claes, M., De Groef, L., and Moons, L. (2022) The DREADDful Hurdles and Opportunities of the Chronic Chemogenetic Toolbox. *Cells* **11**