The blood-brain barrier and beyond: the role of infectious disease in barrier dysfunction

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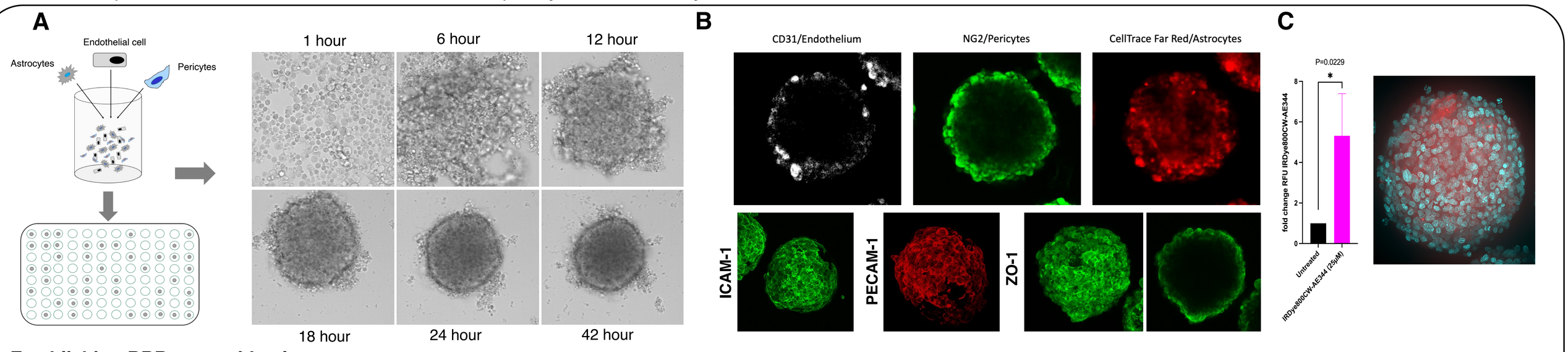
Introduction

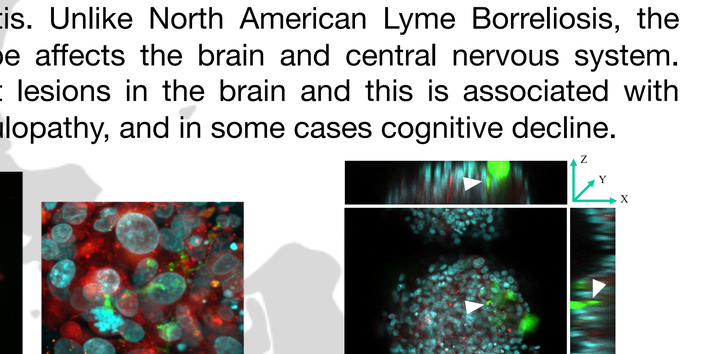
The blood-brain-barrier is a tightly regulated border separating the brain from the circulatory system and restricts the passage of pathogens and toxins, whilst selectively allowing transport of metabolic products i.e. glucose. During cerebral malaria, parasites sequester in large numbers within the cerebral micro vessels and in fatal cases, this culminates in sudden, rapid swelling of the brain[1]. Death can occur due to compression of the brain stem. The exact mechanisms that trigger the development of fatal disease are not fully understood, nor is the impact of the adhesion of specific subsets of parasites on the blood-brain-barrier (BBB). To gain a greater understanding of the impact of parasite adhesion on the BBB, we utilised a recently developed a BBB-organoid model, which recapitulates the interactions between components of the BBB i.e. endothelial cells, pericytes and astrocytes[2].



Establishing BBB-organoid cultures. (A) Schematic and series of brightfield images showing formation of spheroids (0-42 hours). B) Confocal images on organoids stained for component cells CD31 stained endothelial cells, NG2 stained pericytes, while astrocytes were pre-stained prior to assembly with CellTrace Far Red. Organoids express tight-junctions (ZO-1), and receptors such as ICAM-1 and PECAM-1. They are uniform in size and shape and grow between 250-350μm in diameter. C) Originally developed for drug transport studies, they can also be used to test fluorescent surgery probes[3], or investigate the effect of pathogens.

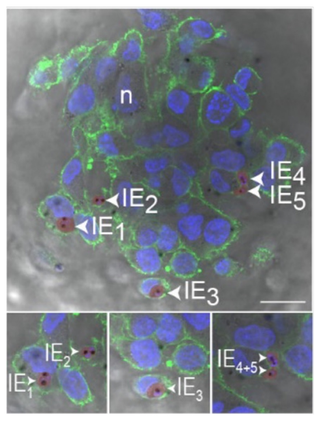
Malaria is transmitted by the bite of an infected mosquito and there are 5 species that can infect humans. *Plasmodium falciparum* is the most wide-spread and infection is frequently fatal. Approx. 60% of the global population lives in areas where malaria is transmitted.

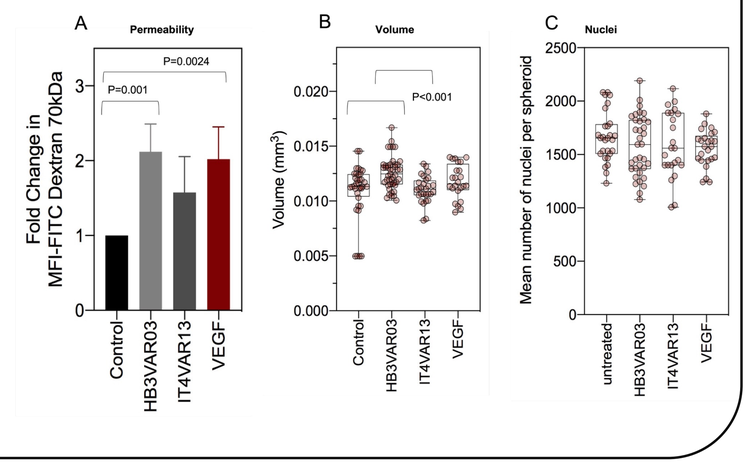
Plasmodium falciparum predominantly affects children <5 years old in sub-Saharan Africa. Parasites invade red blood cells and avoid splenic destruction by binding to the microvasculature. When this occurs in the brain, it's called cerebral malaria. MRI showing a normal scan (A), while (B) is the scan of a child with cerebral malaria showing loss of contrast, diffuse structures and the arrows point towards swollen brain stem and crushed cerebellum.



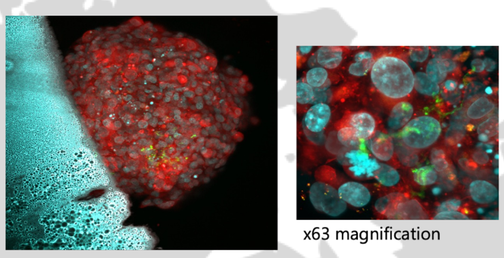
The parasites utilises a large, highly variable protein called PfEMP1 to bind to the micro-vasculature via receptors such as CD36, EPCR, or ICAM-1 (C1, D1)[4]. Those binding both receptors are associated with cerebral malaria and can induce clustering of ICAM-1 (C2), triggering increased adhesion (C3 and 4). Those binding to ICAM-1 and CD36 fail to cluster ICAM-1 (D1 and 2) and binding remains stable over time (D3 and 4)[5].

Parasites selectively alter BBB-integrity. **(A)** Fold change in RFU of FITC-Dx-70kDa after co-incubation with HB3VAR03 or IT4VAR13 IEs compared to untreated controls. VEGF included as positive control for loss of barrier function. (B) Volume measurement of BBB-organoids after exposure to parasites. Significant increase in volume with HB3VAR03 IE exposed, whilst IT4VAR13 IE show no change. VEGF treated organoids failed to show increased volume, even with barrier disruption. (C) Mean number of nuclei per spheroid showing no significant difference between groups.





Lyme Neuroborrelisosis is read by the bite of infected Ixodid ticks. Erythema migraines, or "bullseye" sign is the most common manifestation. Here in Denmark, LNB is the leading cause of bacterial meningitis. Unlike North American Lyme Borreliosis, the infection in Europe affects the brain and central nervous system. MRI scans detect lesions in the brain and this is associated with facial palsy, radiculopathy, and in some cases cognitive decline.



By exposing BBB-organoids to spirochetes isolated from patients who presented with LNB (*B. garinii*), non-LNB (*B. afzelii*), or North American isolates (*B. Burgdorferi* s.s.), we identify organotropism amongst the European isolate *B. garinii*. Exposure to spirochetes results in loss of tight-junctions, reduced integrity, but surprisingly, limited cell death is recorded.

SARS Cov-2 spike protein binds to organoids

BBB-organoids are treated with 3nM or 30nM spike protein from SARS-Cov-2 conjugated to Alexa 488 nanobodies. Organoids were incubated overnight and puncta observed on surface in dose dependant manner.

The spike protein used is part of the SARS-Cov 2 vaccine developed by colleagues at CMP and is now under Phase III clinical trial with Bavarian Nordic.

Sera from convalescent patients can disrupt the blood-brain barrier months after infection

To illustrate the impact of systemic inflammation on the BBB and how it may contribute to dysfunction, BBB-organoids were treated overnight with sera from patients who had been infected with SARS Cov-2. The patients were part of a study into long covid and had reported multiple sequelae months after infection. The graph shows the permeability of organoids exposed to sera from three different patients on day 69, 116, or 139 post-infection.

Conclusions

- **BBB organoids are easy to make**
- **Form a functional BBB**
- **Parasites selectively disrupt the BBB**
- **Cerebral malaria parasite exposed BBB-organoids show significant increase in volume mimicking swelling seen *in vivo***
- **Organoids support investigation of other pathogens**
- **Systemic inflammation can also disrupt the BBB – serious implications for neurodegeneration**

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