Monitoring of Cryptococcus lung infection with IntraGate MRI

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BACKGROUND: *Cryptococcus* is an encapsulated yeast that causes life-threatening disease in both immuno-competent and immuno-suppressed individuals. The two predominant pathogenic strains of this yeast, *Cryptococcus neoformans* and *Cryptococcus gattii*, are generally found in soil, bird excrement and in the bark of certain trees and enter the host via inhalation. Cryptococcosis mostly affects the lung of a host and may spread to the brain, manifesting itself by cryptococcal meningitis and/or pseudocystic lesions (cryptococcoma) in the brain. It remains still unknown why, how and when the cryptococci are able to cross an apparently intact blood-brain barrier (BBB). Histological techniques will remain essential to confirm and unravel cellular and molecular interactions, but imaging techniques are indispensible to define the relevant time frames for histological analysis and this for each animal individually, to investigate crucial events in pathogenesis. As currently imaging tools to evaluate pneumonial cryptococcosis with good temporal and spatial resolution *in vivo* are lacking (for example, µCT is limited because of radiotoxicity concerns), advances made in lung MRI techniques to follow-up disease progression non-invasively will greatly enhance the cryptococcosis research.

AIMS: It is our objective to dynamically monitor cryptococcosis non-invasively in individual animals, in a mouse model for pneumonial and cerebral cryptococcosis. Thereby, we will establish the kinetics of cryptococcal lung infection and the time profile of *Cryptococcus* spreading to the CNS, thereby defining the most critical time points for histological and immunological analysis of key events in the pathogenesis of cryptococcosis.

METHODS: Balb/C mice were infected by inhalation of a *C. gattii* R265 cell suspension (500 cfu's) in PBS (n = 6) or with PBS alone (control, n = 4). Mice were scanned at baseline and at regular time points until 45 days post infection. MRI images were acquired on a horizontal Bruker Biospec (9.4T, 20 cm) in combination with a 7cm quadrature coil using a retrospectively gated FLASH sequence IntraGate (Bruker Biospin, Ettlingen, Germany) with specific parameters: TR/TE = 30/1.26 ms, 17 deg flip angle, 5 slices covering the lung, slice thickness 1 mm and gap of 0.5 mm, FOV = 4 cm x 4 cm, matrix 256 x 256, in plane resolution of 156 µm, 80 repetitions resulting in a 10 min acquisition; the navigator slab was 1cm wide excited with a 0.8 ms sinc10H pulse with a 1.5 deg flip angle. For reconstruction, 70% of the respiration and ECG period was used (Paravision 5.1, Bruker). CT images were acquired on a small animal µCT scanner (SkyScan 1076, Kontich, Belgium) with the following parameters: 50 kV, 0.5 mm Al filter, 200 µA source current, 35 µm isotropic resolution, 120 ms exposure time, 0.7° rotation step and retrospectively gated. Image analysis, segmentation and quantification of CT data were performed with custom written algorithms using SkyScan software. After the last time point, mice were sacrificed followed by sterile CNS removal and lung isolation for histological analysis (HE and PAS-stainings) and quantification of fungal load.

RESULTS: Pneumonial cryptococcosis was successfully and reproducibly induced in immunocompetent mice. While the mice showed no phenotypical signs of cryptococcosis, the progression of the lung pathology could be non-invasively visualized using the here evaluated protocol for IntraGate MRI and was validated using µCT at different time points post infection (see figure). Cryptococcal invasion of the lung was confirmed by histochemical analysis, lung fungal load was quantified and correlated with CT and MRI data (see figure, last panel).

CONCLUSIONS & PERSPECTIVES: To the best of our knowledge, this is the first study showing that non-invasive monitoring of pneumonial cryptococcosis is feasible with retrospectively gated MRI (Intragate, Bruker) resulting in high resolution and contrast images. This MRI approach will allow longitudinal screening of animals, without radiotoxicity concerns as for CT, thereby visualizing infection onset and progression on an individual basis and far before the appearance of any phenotypical signs of disease. We will further finetune the timing of disease onset and correlate this with the time of traversal of *cryptococcus* cells to the CNS. MR imaging of cryptococcosis will greatly help unraveling the still enigmatic pathogenesis of this life-threatening disease.