In vivo MRI and MRA at 9.4T show how (LT) α–TNF receptor 2 or LTβ receptor deficiency on mutant mice affects the development of experimental malaria.

D. TOGBE[1], P. LOUREIRO DE SOUSA[2], M. FAUCONNIER[1], V. BOISSAY[1], L. FICK[3], S. SCHEU[4], K. PFEFFER[4], R. MENARD[5], G. GRAU[6], L. RENIA[7], B. RYFFEL[1], V. QUESNIAUX[1], B-T. DOAN[2,8], and J-C. BELOEIL[2]

[1]University of Orléans, Orléans, France, [2]CBM, CNRS, France, [3]Institute of Infectious Disesase, Cape Town, South Africa, [4]University of Dusseldorf, Germany, [5]Pasteur Institute, Paris, France, [6]The University of Sydney, Australia, [7]Institut Cochin, France, [8]ICSN, CNRS, Gif sur Yvette, France

INTRODUCTION

Cerebral malaria is a frequent cause of death in children and young adults infected with Plasmodium falciparum, which is characterized by the sequestration of parasitized erythrocytes in cerebral blood vessels. In patients with cerebral malaria, disease severity has been correlated with TNF serum levels. In experimental murine malaria the administration of TNF neutralizing antibodies prevented experimental cerebral malaria (ECM) [1]

In this work, investigations on experimental cerebral malaria (ECM) induced *Plasmodium berghei* ANKA (PbA) revealed that the lymphotoxin α (LTα) and signaling through TNFR2 are critical. Indeed, LTβR deficient mice, like LTαβ deficient mice, did not develop the neurological signs seen in PbA induced ECM contrary to C57BL/6J wild type and TNF deficient mice but died at three weeks with high parasitaemia and severe anemia.

MRI techniques (T2 and angiography) were used to verify the lack of ischemia and microvascular pathology in TNF, LTαβ and LTβR deficient malarial mice. The results were validated by histological studies.

MATERIAL AND METHODS

Mice deficient for LTβR(n = 7) or LTαβ(n = 6), and wild-type C57BL/6 or TNF-deficient mice used as ECM sensitive controls, bred at the Transgenose Institute (CNRS, Orleans, France) were examined by MRI at day 7. The 7-10 weeks old mice were infected by intraperitoneal injection of a cloned line of *Plasmodium berghei ANKA* (PbA) with 10^6 parasitized erythrocytes.

MRI experiments were performed in a horizontal 9.4T/21 USR Biospec spectrometer (Bruker Biospin, Wissembourg, France) equipped with 950 mT/m gradient set. In a birdcage coil (Bruker Biospin) with 35mm inner diameter, a custom-built stereotaxic head holder was used to fix the animals. The mice were anaesthetized with isoflurane (1.5-2.5%) in an O_2/N_2O 1:1 mixture. Respiration was monitored and the mice body temperature was kept at 37 ± 0.5 °C throughout the experiment, using warm water circulation.

Preparatory anatomical sagittal MR images were performed using an MSME sequence : FOV = 20x20 mm^2, matrix = 128x128, 1 slice 1 mm thickness. Brain lesions and global changes in tissue structure have been accessed by T2 weighted axial MR images using an MSME sequence : FOV = 17x17 mm2, matrix = 256x192, 37 slices 0.5 mm thickness, experimental time = 16 min. Measurements of vascular cerebral blood flow were performed by MR angiography (MRA) using FLASH sequence: FOV = 14x 14mm2, matrix = 128x128, TR/TE = 30/5 ms, 51 axial slices 0.3 mm thickness, experimental time = 13 min. Angiograms were produced by generating maximum intensity projections (MIP) after interpolating raw data to obtain an isotropic resolution (109 µm) 3. Image analysis and processing were performed with the software ImageJ (NIH, http://rsb.info.nih.gov/ij). Parasitaemia was checked, histology was performed on formaldehyde fixed brains to assess the CNS microvascular obstruction, erythrocyte accumulation in alveoli, thickening of alveolar septae. Vascular leak was assessed using Evan's blue IV injection and absorbance measurements. TCells were quantified by FACS.

RESULTS AND DISCUSSION

LTαβ and LTβR deficient mice showed unaltered MRI and MRA signals, while wild-type C57BL/6 and TNF-deficient mice presented signs of ischemic brain damage and vascular blood flow perturbations upon blood stage PbA infection. By contrast, 4 out of 9 wild-type mice showed altered MRI, observable as a bilateral hyperintense signal at the corpus callosum and external capsule (*Figure 1)*, and a remarkable vascular blood flow perturbations was detected in 4 out of 6 wildtype mice (*Figure 2*).

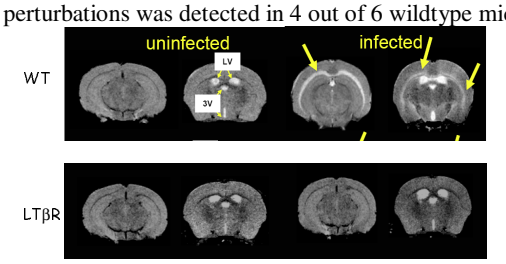

Fig 1 : Wild type and LTβR KO mice brain T2w images, 9.4T

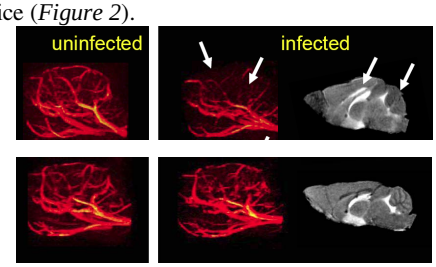

Fig 2 : MR angiograms showing the vascular perturbations in WT mouse

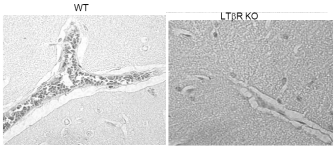

Fig 3 : Microvascular lesions histological images

Resistance of LTαβ or LTβR deficient mice correlated with unaltered cerebral microcirculation and absence of ischemia, as assessed by magnetic resonance imaging and angiography at day 7, associated with lack of microvascular obstruction, while wildtype mice developed distinct and fatal microvascular pathology (*Figure 3*).

We show here that LTβR deficient mice did not develop ECM and survived more than 20 days, while C57BL/6J wild type mice developed typical neurological signs of CM showing that the disruption of LTβR signaling protects from fatal ECM development. Absence of ECM signs in LTβ-R deficient mice correlates with MRI and MRA based morphological analysis. Microscopically, we observed that LTβR deficient mice do not develop microvascular lesions (*Figure 3*), which was completely absent in LTαβ and LTβR deficient mice. FACS revealed that a reduced sequestration of leukocytes in the brain of PbA infected LTβR deficient mice. Parasitaemia is LTαβ-LTβR signaling independent

Finally, absence of LTβR expression on stromal cells determines resistance to ECM

CONCLUSION

We report that absence of LTβR and LTα gene prevented ECM development. Magnetic resonance imaging (MRI) and angiography (MRA) were used to verify the lack of ischemia and microvascular pathology in these mice. The data suggest that both functional LTβR and TNFR2 axis are required for the development of microvascular pathology resulting in fatal ECM. The disruption of either axis prevents cerebral microvascular disease.

REFERENCES

[1] Grau, Get al. 1987.*Science* 237:1210-1212.