

The Meningococcal ABC-Type L-Glutamate Transporter GltT Is Necessary for the Development of Experimental Meningitis in Mice

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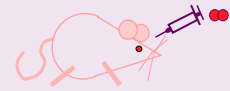
INTRODUCTION

Neisseria meningitidis is a nasopharyngeal coloniser in about 10% of individuals, but sometimes it causes life-threatening diseases, such as meningitis and sepsis. Humans are the only natural hosts for this microbe due to the high specificity of meningococcal surface structures and iron uptake systems for human receptors. Progress in understanding disease pathogenesis and developing effective drugs and vaccines has been hampered by the lack of valuable disease animal models. Two rodent models (mouse and rat) and two infection routes (intraperitoneal and intranasal) are currently employed to induce meningococcal disease (MD). To favor bacterial replication in the murine host, traditional models are based on the use of neonatal subjects, and/or administration of exogenous iron sources, and/or use of large inocula ^{4,7,8,10}. Alternative approaches have been also explored, such as transgenic mice expressing human CD46 ² or transferrin ¹³. To our knowledge, no model of meningococcal meningitis (MM) has been developed in mice infected by the intracranial route. A genome analysis of virulence genes required for MD in the rat showed that about half encode enzymes involved in metabolism and transport of nutrients ¹². Meningococci are auxotrophic for L-glutamate and use it as a nutrient when glucose or lactate are limiting ^{5,6}. Thus, L-glutamate uptake from the host is critical for meningococcal infection ^{5,10}. Moreover, L-glutamate levels are increased in bacterial meningitis and correlate with disease severity ¹¹, and may contribute to neuronal injury ⁹.

METHODS

BACTERIAL STRAINS
MOUSE STRAIN
ROUTE OF INFECTION
INFECTION DOSES
IRON SOURCE
READOUTS

93/4286 (w.t.) and isogenic Ω gltT of group C *N. meningitidis*
8-9 wk-old outbred CD1 female mice
intracranial (i.c.) ¹ or intracisternal (i.cist.) ³
 10^5 - 10^7 CFU/mouse
iron dextran (5mg/kg) i.p. prior to infection
survival and clinical signs, CFU, histology, competitive index (mixed infections)



RESULTS

DEVELOPMENT OF A MM MODEL IN THE MOUSE

1. Comparison of i.c. vs. i.cist. route of infection. Mice were infected with *N. meningitidis* by the i.c. or i.cist. route. No differences in survival and clinical signs of infected mice, and in CFU counts in the blood were observed between animals infected i.c. or i.cist (Fig. 1). In contrast, histological analysis of the brain showed that meningeal and ventricular inflammation and neuronal damage in the hippocampus were more severe in mice infected i.cist. compared to those inoculated i.c. (Fig. 2). Thus, the i.cist. route was chosen for further experiments.

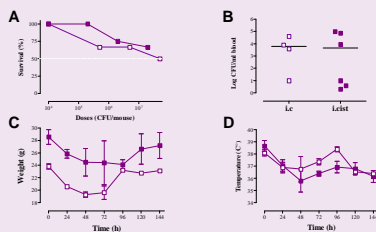


Fig. 1. Analysis of disease induction in mice after i.c. or i.cist. infection with *N. meningitidis*. Animals were infected with the 93/4286 strain by the i.c.(D) or i.cist. (■) route. Mouse survival (A), body weight (C) and temperature (D), and CFU in the blood (B).

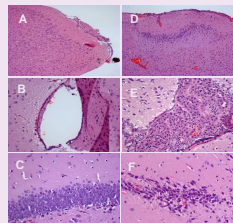


Fig. 2. Histology of brain of infected mice. Mice were infected by the i.c. (A, B, C) or i.cist. (D, E, F) route with the 93/4286 strain. A, D. meninges. B, E. Ventricles. C, F. Hippocampus.

2. Virulence comparison of laboratory-grown vs. mouse-passaged *N. meningitidis*. To evaluate the importance of mouse-passage, naive mice were inoculated by the i.cist. route with either lab-grown or mouse-passaged (i.cist.) 93/4286 meningococci. In the brain, CFU of mouse-passaged bacteria steadily increased over time compared to the lab strain, that did not exhibit logarithmic growth (Fig. 3A). Also, mouse-passaged meningococci efficiently replicated in the spleen and liver (Fig. 3C-D). On the contrary, the lab strain was cleared systemically within 48h from infection (Fig. 3B,C,D). Mouse-passage is a key feature of the MM model.

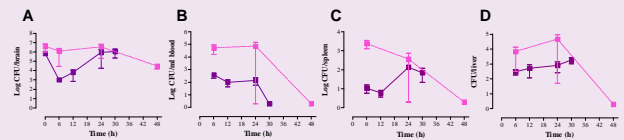


Figure 3. CFU counts after i.cist. infection with *N. meningitidis* 93/4286. Animals were infected by the i.cist. route with either lab-grown (■) or mouse-passaged (●) meningococci. For passage, bacteria were used to infect mice by i.cist. injection and recovered 24h from homogenised brains. Rodents were sacrificed at various time points after infection, and CFU counts were performed in the brain (A), blood (B), spleen (C), and liver (D).

ROLE OF THE GLUTAMATE TRANSPORTER GltT IN MURINE MM

3. Virulence analysis of the Ω gltT mutant in MM. A 93/4286 isogenic mutant deficient in the GltT transporter was constructed. The w.t. and Ω gltT strains were assessed in the MM mouse model. Data on survival and clinical signs indicated moderate mutant attenuation, but differences were poorly significant (Fig. 4). In contrast, analysis of bacterial loads in organs showed virulence reduction of the Ω gltT strain (Fig. 5). In the brain, CFU of the mutant at 24h were ~ 400-folds lower than those of the w.t., that instead persisted at the infection site reaching ~6.7 log CFU (Fig. 5A).

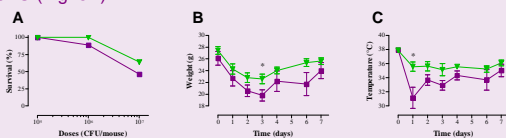


Fig. 4. Mouse survival and clinical signs after meningococcal infection. Mice were infected i.cist. with 10^7 CFU of the w.t. (■) or Ω gltT mutant (▼). Mouse survival (A), body weight (B) and temperature (C).

Systemically, the mutant was severely hampered and was cleared within 24h, whereas the w.t. could still be recovered at high numbers (Fig. 5B,C,D).

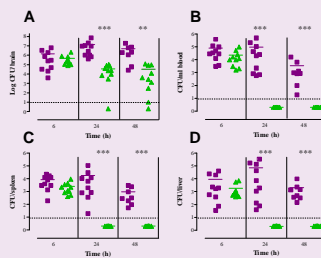


Fig. 5. CFU counts in organs of infected mice. Mice were infected with 10^7 CFU of the w.t. (■) or the Ω gltT mutant (▼). CFU were counted over time in brain (A), blood (B), spleen (C), and liver (D).

4. The Ω gltT mutant is less fit than the w.t. in i.cist. coinfection. In competition experiments, the w.t. outgrew the mutant in all organs and time-points (Fig. 6). Already 6h post-challenge, CIs were as low as 0.14 in the brain and 0.02 systemically. At later stages, CIs progressively decreased to 0.007 in the brain (Fig. 6A), 0.004 in spleen and liver (Fig. 6C-D), down to 0.0002 in the blood (Fig. 6B).

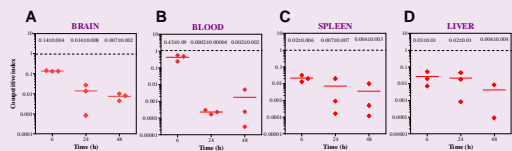


Fig. 6. Competition of Ω gltT and w.t. meningococci in MM. Mice were coinfectd i.cist. with 10^7 CFU of both strains (1:1). Six, 24 and 48h later, brain (A), blood (B), spleen (C), and liver (D) were plated with and without antibiotic to distinguish between mutant and w.t., respectively. Competitive Index (CI, ●) of single animals over time and mean (bars) \pm SEM of CI are shown. CI < 1 indicates reduced fitness of the Ω gltT strain.

CONCLUSIONS

- ✓ Development of a novel and effective murine MM model based on i.cist. infection and mouse-passage
- ✓ The GltT transporter is attenuated in virulence in mouse MM, indicating that glutamate uptake is crucial for meningococcal replication and survival in the murine host.
- ✓ In MM, meningococci may use L-glutamate as a nutrient source and antioxidant (glutathione synthesis)
- ✓ Potential to use L-glutamate antagonists to halt bacterial replication and block L-glutamate neurotoxicity

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