

4.5. Animal studies

Female C57BL/6 mice aged at 6 weeks were purchased from CLEA Japan (Tokyo, Japan). All mice were kept under specific pathogen free conditions in animal facility of Osaka City University Graduate School of Medicine according to the institutional guidelines for the animal experiments. Twenty mice were used per group for infecting with each strain. One hundred microlitre of bacterial suspension containing 1×10^5 CFUs of MAC was inoculated into the trachea of the 7 weeks-aged mice anesthetized with pentobarbital sodium. Lungs, spleens and livers were removed on day 1 (only lungs) and 4, 8, 16 weeks after inoculation from 5-mice per strain. The organs were homogenized in 1 ml saline, and 0.1 ml of 10-fold dilutions of the homogenates was plated on 7H11-OADC agar followed by cultivating for 3 weeks. Bacterial burden was evaluated by CFUs per organ. Histological sections were made by standard methods including formalin fixation, dehydration, embedding in paraffin, and staining with hematoxylin and eosin.

4.6. Statistical analysis

Data were analyzed using the statistical analysis software package StatView 5.0 (SAS Institute, Cary, NC). The difference of mycobacterial growth in 7H9 broth, THP-1 cells, and mice was compared by a post hoc test of Scheffé among the strains tested. The difference of mycobacterial growth at defined time points during infection in THP-1 cells as well as in mice was compared by repeated measurement ANOVA with a post hoc test of Scheffé in the individual strains. Difference was considered statistically significant at $P < 0.05$.

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