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An optimized cyclophosphamide-treated mouse model of *Mycobacterium abscessus* pulmonary infection

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ABSTRACT *Mycobacterium abscessus* pulmonary infections are increasingly problematic, especially for immunocompromised individuals and those with underlying lung conditions. Currently, there is no reliable standardized treatment, underscoring the need for improved preclinical drug testing. We present a simplified immunosuppressed mouse model using only four injections of cyclophosphamide, which allows for sustained *M. abscessus* lung burden for up to 16 days. This model proved effective for antibiotic efficacy evaluation, as demonstrated with imipenem or amikacin.

KEYWORDS *M. abscessus*, mouse model, lung infection

Mycobacterium abscessus, a rapid-growing nontuberculous mycobacterium, causes lung, skin, and soft tissue infections (1). *M. abscessus* pulmonary infections are now a major health concern, particularly among immunodeficient patients or those with underlying lung conditions such as bronchiectasis or cystic fibrosis (2–4). *M. abscessus* resistance to many drugs makes eradication challenging (5–8). Due to the low cure rate of current therapies (9), there is a great interest in developing new drugs and regimens to treat *M. abscessus* lung infections. Unfortunately, many drugs with promising *in vitro* potency fail to translate to clinical efficacy (10). Hence, preclinical animal models are vital. While mouse models are common for infection research and drug testing, developing a model for *M. abscessus* is challenging due to its opportunistic nature. Immunocompetent mouse strains eliminate infection quickly (11–14), whereas certain genetically altered strains such as nude, NOD SCID, or GM-CSF knock-out maintain high bacterial counts (11, 14–17), but are costly. Pharmacological treatment with dexamethasone allows *M. abscessus* to persist in mice but requires daily injections (13). We present here a simplified model with cyclophosphamide-treated BALB/c mice, which requires only four injections and offers a cost-effective method for antibiotic testing.

Since our initial goal was to use a simple and reliable model to test antibiotic efficacy in a mouse model of *M. abscessus* lung infection, we first evaluated cyclophosphamide treatment as previously described (18). Seven-week-old female BALB/c mice received intraperitoneal injection of 150 mg/kg cyclophosphamide 4 days and 1 day prior to intranasal infection with 1.0×10^7 CFU of the *M. abscessus* reference strain CIP104536 (rough variant). Following euthanasia, lungs were harvested, homogenized, serially diluted, and plated on Middlebrook 7H11 selective agar at 37°C for CFU enumeration. Our results showed that the bacterial burden in the lungs increased from 6.7 $\log_{10}$ CFU/lung on day 1 to 8.7 $\log_{10}$ CFU/lung by day 7. However, the mice rapidly appeared moribund, necessitating early termination of the experiment. At the time of sacrifice on day 11, the lung bacterial burden had declined to day 1 levels, indicating that the decline in the mice's health was due to cyclophosphamide-induced toxicity rather than uncontrolled bacterial proliferation. Lowering the cyclophosphamide doses to 100 mg/kg was better tolerated. However, bacterial lung burden dropped sharply by 2.4

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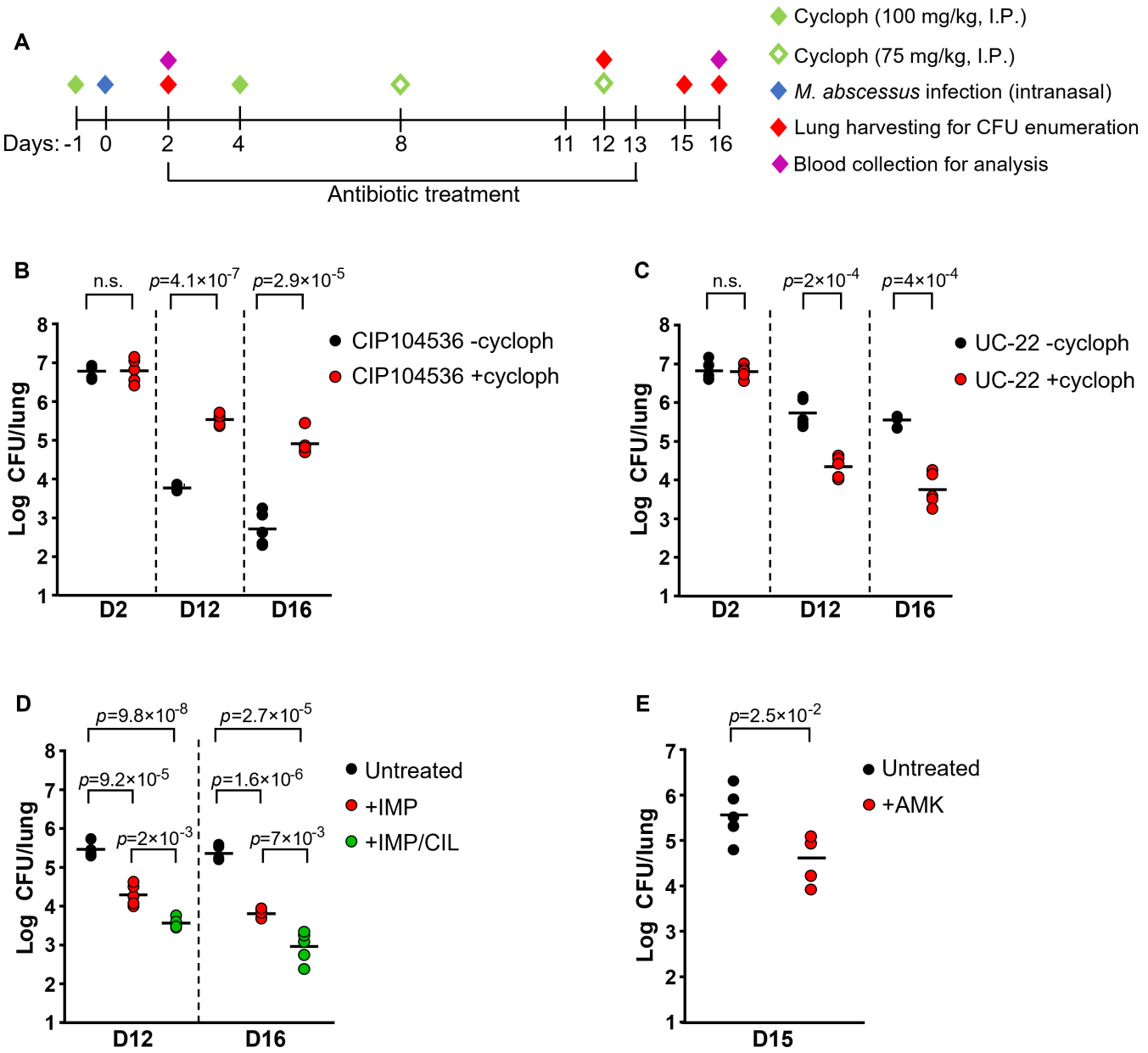

FIG 1 A cyclophosphamide-treated mouse model of *M. abscessus* infection suitable for the evaluation of drug efficacy. (A) Schematic of experimental procedure. (B) *M. abscessus* CIP104536 (R) lung burdens in mice with and without cyclophosphamide treatment. (C) *M. abscessus* UC-22 lung burdens in mice with and without cyclophosphamide treatment. (D) *M. abscessus* CIP104536 (R) lung burdens in immunosuppressed mice treated twice daily for 10 days with either 100 mg/kg imipenem, 100 mg/kg imipenem and cilastatin (1:1 dose ratio), or untreated. (E) *M. abscessus* CIP104536 (R) lung burdens in immunosuppressed mice treated daily for 12 days with 150 mg/kg amikacin (AMK) or untreated. Student *t*-test was performed to calculate the *p*-values. Cycloph: cyclophosphamide; IMP: imipenem; CIL: cilastatin; AMK: amikacin; n.s: not significant. The results were repeated at least once.

orders of magnitude between day 1 and day 14. These results highlighted the problems with the current dosing scheme: higher drug doses could not be tolerated by the mice, while a lower dose failed to maintain a steady bacterial lung burden over time.

To address these limitations, we devised a new scheme in which 4 doses of pharmaceutical-grade cyclophosphamide (Endoxan®, Baxter) were administered via intraperitoneal injection: two doses of 100 mg/kg 1 day before infection and on day 4 post-infection, followed by two more doses of 75 mg/kg on day 8 and day 12 post-infection, to sustain immunosuppression (Fig. 1A). All mice were infected intranasally with

1×10^7 CFU of *M. abscessus* CIP104536 (R) and divided into two groups: (i) a control group that did not receive any cyclophosphamide and (ii) a group that received the four doses. Blood was collected on day 2 and day 16 post-infection for hematological analysis. Analysis revealed a decrease in immune cells on day 2 that was sustained until day 16 (Table S1). On day 2 post-infection, the bacterial burden was similar in the untreated and cyclophosphamide-treated groups (Fig. 1B). However, the bacterial burden in immunocompetent animals decreased by three orders of magnitude between day 2 and day 12, with an additional ~1.06 $\log_{10}$ decrease from day 12 to day 16 (Fig. 1B), which was consistent with the literature (13, 16). In the group that received cyclophosphamide, the bacterial burden decreased more modestly by ~1.26 orders of magnitude from day 2 to day 12 and remained high, ~4.9 Log_{10} CFU/lung, on day 16 (Fig. 1B). These results indicate that the immunosuppression protocol allows for sustained lung colonization while being well-tolerated. Histopathological analyses revealed sustained inflammatory phenotype with neutrophilic and macrophage infiltration, resembling persistent bacterial infection (Fig. S1), indicating some degrees of immunological response despite immunosuppression. Furthermore, we tested whether this model was also suitable for the study of other clinical isolates (19). The same cyclophosphamide treatment scheme (Fig. 1A) also allowed long-term lung colonization with the clinical isolate UC-22 (19) that was otherwise rapidly cleared (Fig. 1C). Together, these results indicate that the protocol is appropriate to allow maintenance of a high level of bacterial lung burden for up to 16 days.

Next, we examined whether the model is suitable for the evaluation of antibiotic efficacy. We chose imipenem and amikacin as reference drugs. Imipenem was given alone at 100 mg/kg as reported before (20) or in combination with cilastatin (1:1) at 100 mg/kg to reduce the rapid metabolization of imipenem in rodents (21). Treatment was initiated 2 days post-infection, twice daily for 10 days. Imipenem alone reduced lung bacterial burden by ~90 and ~99% when given alone or in combination with cilastatin, respectively (Fig. 1D). Amikacin at 150 mg/kg once a day (20) for 12 days also caused an ~90% reduction in bacterial burden (Fig. 1E).

In summary, we describe here a model of *M. abscessus* pulmonary infection, which is suitable for evaluating drug efficacy. One of the limitations of the study is the use of antibiotic at doses not reflecting human drug blood levels. An in-depth analysis of the immune response also remains to be studied. Furthermore, the model may not reflect the immune response experienced in patients with bronchiectasis or cystic fibrosis. Although no single animal model can comprehensively recapitulate all aspects of *M. abscessus* pulmonary infection and pathology in humans, the simple model described in this study is a valuable and cost-effective addition to *M. abscessus* preclinical studies.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental material (AAC01520-23-s0001.docx). Tables S1 and S2; Fig. S1.

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