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Prophylactic efficacy of a human monoclonal antibody against MERS-CoV in the common marmoset

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**ABSTRACT**

Effective antiviral treatments for MERS-CoV are urgently needed. LCA60 is a MERS-CoV-neutralizing monoclonal antibody isolated from a convalescent MERS patient. Previously, it was shown that treatment with LCA60 resulted in reduced disease and virus titers in mouse models of MERS-CoV infection. Here, we tested the prophylactic efficacy of LCA60 in the common marmoset model of MERS-CoV infection. Intravenous administration of LCA60 one day before virus challenge resulted in high levels of MERS-CoV-neutralizing activity in circulating blood. Clinically, there was a moderate benefit of treatment with LCA60 including reduced respiratory involvement. Although viral lung loads were not reduced in LCA60-treated animals as compared to controls, there were fewer pathological changes in the lungs. Thus, prophylactic LCA60 treatment could be implemented to reduce disease burden in contacts of confirmed MERS-CoV patients.

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) can cause severe respiratory disease with a high case-fatality rate in humans. There are currently no approved treatments for MERS. LCA60 is a human IgG1 monoclonal antibody that was isolated from the memory B cells derived from a convalescent MERS patient in the UK (Bermingham et al., 2012; Corti et al., 2016) that neutralizes MERS-CoV by binding to the receptor binding domain of the MERS-CoV spike protein (Corti et al., 2015). Balb/c mice transiently expressing human DPP4 in lung tissue after transduction with an adenovirus vector expressing human DPP4 and treated with LCA60 one day before or after challenge with MERS-CoV showed a significant reduction of virus titers in the lungs compared to controls (Corti et al., 2015). In hDPP4-transduced IFNAR-KO mice, treatment with LCA60 one day after inoculation with MERS-CoV resulted in reduced disease, as indicated by bodyweight loss, as well as a significant reduction in lung virus titers (Corti et al., 2015). These promising results prompted us to test the prophylactic efficacy of LCA60 treatment in a distinct animal model of MERS, the common marmoset, to support licensure for human use. In contrast to mice, common marmosets are naturally susceptible to MERS-CoV and develop moderate to severe disease upon infection (Falzarano et al., 2014).

All animal experiments were approved by the Institutional Animal Care and Use Committee of Rocky Mountain Laboratories, NIAID, NIH and carried out by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility, according to the institution's guidelines for animal use, and followed the guidelines and basic principles in the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals. All infectious work with MERS-CoV was approved by the Institutional Biosafety Committee and performed in a high containment facility at RML. Sample inactivation was performed according to standard operating procedures for removal of specimens from high containment approved by the Institutional Biosafety Committee.

Twelve common marmosets (Callithrix jacchus; 2–6 years old) were randomly assigned into two groups of six animals. Animals in the first group (5 males; 1 female) were intravenously infused with 30 mg/kg of mAb LCA60; those in the second group (4 males, 2 females) were intravenously infused with MPE8, a human monoclonal antibody that neutralizes human respiratory syncytial virus (hRSV) and was used as a
Monoclonal antibodies were infused into the femoral vein in a volume of ≤1 ml depending on bodyweight. MERS-CoV-neutralizing activity in serum was determined at several time-points after infusion using a microneutralization assay with 100 TCID\textsubscript{50} hCoV-EMC/2012 as described previously (de Wit et al., 2018). Serum samples collected on 1, 3 and 7 days after administration of LCA60 contained high levels of MERS-CoV neutralizing activity; no MERS-CoV neutralizing antibodies were detected in the control mAb-treated animals (Fig. 1A). Although neutralizing titers in the serum of LCA60-treated animals dropped between the first and last measurement, reciprocal serum neutralizing titers remained high throughout the experiment in all animals (range 480–1920; Fig. 1A). Twenty-four hours after administration of neutralizing antibody preparations, all animals were euthanized and tissue samples were collected from all 4 lung lobes, RNA was extracted, qRT-PCR targeting UpE performed, and viral load determined as TCID\textsubscript{50} equivalents per gram tissue as described previously (de Wit et al., 2018).
2014). Scoring was done by the same individual throughout the study; this person was blinded to assignment of the animals to the control mAb- or LCA60-treated group. Regardless of treatment, all animals showed signs of disease after inoculation with MERS-CoV; however, disease scores appeared lower in the animals treated with LCA60, although this difference was statistically significant only on 2 days post infection (dpi) (Fig. 1B). Clinical exams were performed on anesthetized animals on 0, 2, 4 and 6 dpi during which bodyweight and respiratory rate were determined and chest radiographs were taken. All animals lost bodyweight after inoculation with MERS-CoV; on 6 dpi the bodyweight loss observed in the LCA60-treated animals was statistically significantly less than that in the control animals (Fig. 1C). The animals treated with control mAb all showed increased respiratory rates whereas only 5 of 6 animals treated with LCA60 showed increased respiratory rates. Respiratory rates were significantly higher in the control mAb-treated animals than the LCA60-treated animals on 2 dpi, but not on 4 or 6 dpi (Fig. 1D). Chest radiographs obtained on 6 dpi from all animals were analyzed for the presence of pulmonary infiltrates by a clinical veterinarian blinded to the group assignment of the animals and showed that LCA60-treated animals had significantly lower cumulative scores than control mAb-treated animals (Fig. 1E), indicating that LCA60-treated animals had fewer pulmonary infiltrates and suggesting that respiratory disease was less severe in LCA60-treated animals. One of the common marmosets in the control group reached the established humane endpoint criteria on 6 dpi, the final day of the experiment, with severe respiratory signs, a clinical score of 35 and severe hypothermia (body temperature 33.9 °C, 5.6 °C below baseline; data not shown).

On 6 dpi, all animals were euthanized to determine viral loads and assess pathologic changes in various tissues. Tissues of the respiratory tract, including samples of all four lung lobes, were collected for analysis. RNA was extracted from 30 mg tissue and used in a one-step real-time RT-PCR (qRT-PCR) detecting upE (Corman et al., 2012) as described previously (de Wit et al., 2018). There were no statistical differences in viral loads in LCA60-treated versus control mAb-treated animals (Fig. 1D). Viral loads were determined by qRT-PCR as virus titration in the presence of neutralizing antibody would be hard to interpret since neutralizing antibodies would still be present in the tissue when titration assays are performed, and previously unbound antibody could potentially neutralize virus during titration, making negative findings difficult to interpret. Upon necropsy, the area of lung lobes affected by gross lesions was estimated by a board-certified veterinary pathologist who was blinded to the group assignment of the animals. Although gross lung lesions were observed in all LCA60-and control mAb-treated animals, a statistically significantly smaller area of the lung was affected by lesions in LCA60-treated animals than control-mAb-treated animals (Fig. 2A). Lung weights were determined at necropsy to calculate the lung weight to bodyweight ratio as an indicator of pneumonia; this ratio was significantly lower in the LCA60-treated animals than in the controls (Fig. 2B). Histological assessment of lung tissue by a board-certified veterinary pathologist blinded to group assignment of the animals, showed that all animals except one animal in the LCA60-treated group developed the typical multifocal to coalescing, acute but transient disease that can be fatal in common marmosets. Consideration of this model here due to the moderate clinical benefit observed upon prophylactic treatment, but has been shown in a mouse model of MERS-CoV infection (Corti et al., 2015). However, therapeutic efficacy of several MERS-CoV neutralizing antibodies has been shown in mouse models (Agrawal et al., 2016; Corti et al., 2015; Li et al., 2015; Pascal et al., 2015; Qiu et al., 2016; Zhao et al., 2018), but so far the clinical benefit in nonhuman primate studies has been modest (Chen et al., 2017; de Wit et al., 2018; van Doremalen et al., 2017). Thus, LCA60 would likely be more efficacious as a therapeutic treatment in combination with other MERS-CoV-neutralizing antibodies or other antiviral treatments such as ribavirin, IFN and lopinavir that have shown therapeutic efficacy in nonhuman primates (Chan et al., 2015; Falzarano et al., 2019). A limitation of this study is reflected by the debate on the value of animal disease models for MERS (reviewed in (Baseler et al., 2016)). Neither the mouse nor the nonhuman primate models represent all hallmarks of human MERS. Rodents lack natural susceptibility to MERS-CoV which is overcome by expressing the human receptor by different means. The nonhuman primate models utilize high infectious doses and partial artificial inoculation routes leading to immediate acute but transient disease that can be fatal in common marmosets. Currently, there are insufficient data to address the value of one model over the other (reviewed in (Baseler et al., 2016)). Therefore, treatment efficacy data in both rodent and nonhuman primate models need to be considered and interpreted carefully as they may not necessarily reflect the outcome in humans. Until further evaluation, both these models seem needed to select treatment modalities prior to human clinical trials and thus are important tools for MERS countermeasure development.

Conflicts of interest

EdW, FF, EvH, AO, ELH, GS, DS, RG, MZ and HF have no conflicts to declare.

EC and DC are employees of Vir Biotechnology and hold shares in Vir Biotechnology.

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Fig. 2. Pathological findings in the lungs of common marmosets inoculated with MERS-CoV and treated prophylactically with LCA60. Two groups of six common marmosets were intravenously infused with 30 mg/kg control mAb (black) or MERS-CoV neutralizing mAb LCA60 (red) and inoculated with MERS-CoV 24 h later. On 6 dpi all animals were euthanized and the area of the lung lobes affected by gross lesions was scored by a board-certified veterinary pathologist blinded to treatment received by the animals (A); lungs were weighed to determine the lung weight/bodyweight ratio (B) and samples were collected for histological analysis. Tissues were stained with hematoxylin and eosin (HE) or a rabbit polyclonal α-MERS-CoV antibody (IHC). One representative image was chosen for each group of control mAb-treated and LCA60-treated animals (C). Slides of all four lung lobes of all animals stained with a polyclonal α-MERS-CoV antibody were digitized and antigent-positive pixels were quantified using the ImageScope pixel positive pixel algorithm. The percentage antigen-positive pixels was calculated as the number of pixels stained for MERS-CoV antigen divided by the total number of stained pixels (i.e. non-stained areas such as air spaces were excluded from the analysis) (D). Asterisks indicate statistical significance determined using an unpaired t-test with multiple comparisons using the Holm-Sidak method; ***: p < 0.001.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.01.016.

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