#### S.A., M.F., C.J.L., N.A.B., and L.V. contributed new reagents/analytic tools; D.C., J.Z., M.P., L.S., S.A., M.F., R.G., and L.V. analyzed data: and D.C., J.Z., F.S., R.S.B., L.V., M.Z.,

limited for SARS-CoV (from November 2002 to July 2003) and

long-lasting for MERS-CoV, which appeared in 2012 and con-

CoV probably originated in bats (3, 4) with dromedary camels

serving as intermediate hosts for the human MERS-CoV in-

fection and palm civets and raccoon dogs for SARS-CoV (5).

Dromedary camels have a close association with humans in the

affected areas. Of note, whereas in humans MERS-CoV infects

the lower respiratory tract, rendering human-to-human transmission

inefficient, in camels the virus infects the upper respiratory tract and

is present in nasal secretions at high concentrations, which favors

transmission to humans and other camels. However, the mechanisms

of transmission from camels to humans and from humans to humans

as well as the global incidence in humans are still unclear. Camels

show no or mild symptoms, and antibodies found in banked sera

samples show that the virus has been present in the animals for at

least the past 20 y (6). In addition, most of the patients described

appeared to have been infected in hospitals, from other MERS

Middle East Respiratory Syndrome coronavirus (MERS-CoV) cau-

ses severe respiratory disease with a high mortality rate. There is

no licensed vaccine or antiviral for MERS. Here we isolated for the first time, to our knowledge, a potent MERS-CoV-neutralizing

antibody from memory B cells of an infected individual. This

antibody binds to a novel site on the viral Spike protein, neu-

tralizes by interfering with the binding to the cellular receptor

CD26, and is highly effective both in prophylaxis and in therapy

in a relevant mouse model. This antibody can be developed for

prophylaxis, for postexposure prophylaxis, or for the treatment

Author contributions: D.C., J.Z., F.S., R.S.B., L.V., M.Z., S.P., and A.L. designed research; D.C., J.Z., M.P., L.S., S.A., C.F., B.F.-R., G.A., F.V., and R.G. performed research; M.P., L.S.,

As to the zoonotic reservoir, both MERS-CoV and SARS-

tinues to circulate in the Middle East.

This article is a PNAS Direct Submission.

of severe MERS-CoV infections.

**Significance** 



CrossMark

# Prophylactic and postexposure efficacy of a potent human monoclonal antibody against MERS coronavirus

Davide Corti<sup>a,b,1</sup>, Jincun Zhao<sup>c,d,1</sup>, Mattia Pedotti<sup>a</sup>, Luca Simonelli<sup>a</sup>, Sudhakar Agnihothram<sup>e</sup>, Craig Fett<sup>e</sup>, Blanca Fernandez-Rodriguez<sup>a</sup>, Mathilde Foglierini<sup>a</sup>, Gloria Agatic<sup>b</sup>, Fabrizia Vanzetta<sup>b</sup>, Robin Gopal<sup>f</sup>, Christopher J. Langrish<sup>9</sup>, Nicholas A Barrett<sup>9,h</sup>, Federica Sallusto<sup>a</sup>, Ralph S. Baric<sup>e,i</sup>, Luca Varani<sup>a</sup>, Maria Zambon<sup>f</sup>, Stanley Perlman<sup>c</sup>, and Antonio Lanzavecchia<sup>a,j,2</sup>

<sup>a</sup>Immune Regulation Unit, Institute for Research in Biomedicine, Università della Svizzera Italiana, 6500 Bellinzona, Switzerland; <sup>b</sup>Humabs BioMed SA, 6500 Bellinzona, Switzerland; <sup>c</sup>Department of Microbiology, University of Iowa, Iowa City, IA 52240; <sup>d</sup>State Key Laboratory of Respiratory Diseases, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China; <sup>e</sup>Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Nc 27599; <sup>f</sup>Microbiology Services Colindale, Public Health England, London NW9 5HT, United Kingdom; <sup>g</sup>Guy's and St. Thomas' National Health Service Foundation Trust, London SE1 7EH, United Kingdom; <sup>h</sup>King's College London, Strand, London WC2R 2LS, United Kingdom; <sup>j</sup>Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, Nc 27599; and <sup>j</sup>Institute of Microbiology, Eidgenössische Technische Hochschule Zurich, 8093 Zurich, Switzerland

Edited by Michael B. A. Oldstone, The Scripps Research Institute, La Jolla, CA, and approved June 29, 2015 (received for review May 25, 2015)

Middle East Respiratory Syndrome (MERS) is a highly lethal pulmonary infection caused by a previously unidentified coronavirus (CoV), likely transmitted to humans by infected camels. There is no licensed vaccine or antiviral for MERS, therefore new prophylactic and therapeutic strategies to combat human infections are needed. In this study, we describe, for the first time, to our knowledge, the isolation of a potent MERS-CoV-neutralizing antibody from memory B cells of an infected individual. The antibody, named LCA60, binds to a novel site on the spike protein and potently neutralizes infection of multiple MERS-CoV isolates by interfering with the binding to the cellular receptor CD26. Importantly, using mice transduced with adenovirus expressing human CD26 and infected with MERS-CoV, we show that LCA60 can effectively protect in both prophylactic and postexposure settings. This antibody can be used for prophylaxis, for postexposure prophylaxis of individuals at risk, or for the treatment of human cases of MERS-CoV infection. The fact that it took only 4 mo from the initial screening of B cells derived from a convalescent patient for the development of a stable chinese hamster ovary (CHO) cell line producing neutralizing antibodies at more than 5 g/L provides an example of a rapid pathway toward the generation of effective antiviral therapies against emerging viruses.

MERS CoV | neutralizing antibody | serotherapy | emerging viruses

iddle East Respiratory Syndrome coronavirus (MERS-CoV) is an emergent subgroup C betacoronavirus that was detected for the first times in June and September of 2012, when two cases of severe infections were identified in the Eastern Mediterranean region (1, 2). As of June 29, 2015, 1,379 human infections with 531 deaths have been confirmed in 26 countries in the Middle East, Europe, North Africa, Asia, and Americas, including the recent outbreak in South Korea caused by an individual who traveled to the Middle East, which caused 164 infections and 23 deaths (www.ecdc.europa.eu). MERS-CoV causes severe infection of the lower respiratory tract, similar to the Severe Acute Respiratory Syndrome CoV (SARS-CoV) that appeared in China in 2002. Several cases of human-to-human transmission have been reported in health care workers and family clusters, but at the current time there is no evidence of sustained human-to-human transmission.

SARS-CoV and MERS-CoV belong to the B and C betacoronavirus lineages and have been shown to bind to cellular receptors ACE2 and CD26 [also known as dipeptidyl peptidase 4 (DPP4)], respectively. Of note, SARS-CoV targets ciliated bronchial epithelial cells and type I and type II pneumocytes, whereas MERS-CoV infects type II pneumocytes and nonciliated bronchial cells. These differences might account for the different rates of human-to-human transmission, which was high for SARS-CoV and is moderate to low for MERS-CoV. The two viruses differ also in the duration of their epidemic, which was

M.P., L.S., S.A., M.F., R.G., and L.V. analyzed data; and D.C., J.Z., F.S., R.S.B., L.V., M.Z., S.P., and A.L. wrote the paper.

Conflict of interest statement: A.L. is the scientific founder of Humabs BioMed SA. A.L. holds shares in Humabs BioMed SA. G.A., F.V., and D.C. are employees of Humabs Biomed, a commercial company that commercializes human monoclonal antibodies.

Freely available online through the PNAS open access option.

See Commentary on page 10082.

<sup>&</sup>lt;sup>1</sup>D.C. and J.Z. contributed equally to this work.

<sup>&</sup>lt;sup>2</sup>To whom correspondence should be addressed. Email: lanzavecchia@irb.usi.ch.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10. 1073/pnas.1510199112/-/DCSupplemental.

patients, and even many of those who became infected outside a hospital report no exposure to camels. Recent epidemiological data suggest that more than ~45,000 people in Saudi Arabia were seropositive for a MERS infection, implying that the majority of infections may not detected, the case fatality rate is lower than current estimates of about 40%, or significant levels of unreported severe disease have been misdiagnosed over the past 5 20 y.

The trimeric S protein of MERS-CoV mediates receptor binding and membrane fusion and is the major target for neutralizing antibodies (7, 8). The structure of the cellular receptor (CD26), which is conserved across many species (9), and the complex of CD26 with the receptor binding domain (RBD) of the S protein have been determined by X-ray crystallography (10, 11).

Recently, two groups have used nonimmune human antibodyphage display libraries to isolate neutralizing antibodies to MERS-CoV (12, 13). The antibody 3B11 described by Tang et al. (12) showed neutralizing activity against some but not all strains. m336, a second antibody described more recently by Ying et al. (13), showed high neutralizing potency and used the same VH gene (i.e., VH1-69) with high homology to 3B11 in the HCDR3, suggesting that the two antibodies might have a similar mode of interaction. This hypothesis is supported by the analysis of m336 escape mutants. Here we describe for the first time, to our knowledge, the isolation of a potent neutralizing human monoclonal antibody from the memory B cells of an infected patient that might be developed for the prevention and treatment of MERS-CoV infections.

### Results

Rapid Isolation and Scaled-Up Production of a Potent MERS-CoV Human Neutralizing Antibody. A 49-y-old man from Qatar (14) was hospitalized with severe respiratory failure, requiring extracorporeal membrane oxygenation for over 200 d at St. Thomas's Hospital in London. Diagnosis was established by RT-PCR detection of corona virus, which was found to be a novel virus (14), subsequently isolated, and thereafter defined as London1/2012 strain. Serum samples and mononuclear cells were obtained at two time points. A pseudotyped luciferase-based MERS-CoV neutralization assay was developed using the sequence of the S protein of the autologous virus (i.e., London1/2012). The donor serum samples obtained 5 and 8 mo after infection showed only modest neutralizing activity (Fig. 1A), a finding that illustrates the difficulty of obtaining neutralizing antibodies for passive serotherapy from infected patients. IgG memory B cells were immortalized under clonal conditions, and supernatants were screened using a pseudotyped neutralization assay and an ELISA on fixed MERS-CoV infected cells (Fig. 1B).

A single B-cell culture out of ~4,600 showed neutralizing activity, indicating a very low frequency of MERS-CoV specific B cells, which is consistent with the low serum neutralizing titer. The antibody isolated from this culture, dubbed LCA60, showed an IC50 value of 10 ng/mL against the London1/2012 MERS-CoV pseudovirus (Fig. 1C). We also isolated a nonneutralizing antibody (dubbed LCA57) from the ELISA screening. Both LCA60 and LCA57 bound to the S protein from the EMC strain, as determined by ELISA (Fig. 1D), but not to the S protein of HKU4.2 (group IIC, 67% amino acid identity) and HKU3 (group IIB, 30% amino acid identity). LCA60 was confirmed to neutralize, with high and comparable efficiency, infectious MERS-CoV from the London patient as well as additional isolates obtained from Saudi Arabia and Jordan (IC50 values, 279 ng/mL for London1/2012, 150 ng/mL for EMC/2012, and 110 ng/mL for the Jordan-N3/2012 isolate) (Fig. 1E). These results indicate that LCA60 is 10-fold more potent than 3B11 and comparable to m336. The binding rate constants were determined by surfaceplasmon resonance (Fig. 2). LCA60 showed subnanomolar affinity for the S protein [equilibrium dissociation constant (KD) equivalent to 0.12 nM], which was ~300-fold higher compared with



**Fig. 1.** Isolation of the LCA60 antibody from the memory B cells of a MERS CoV infected individual. MERS survivor plasma samples (two time points, T1 and T2, corresponding to 5 and 8 mo after infection, respectively) (*A*) and supernatants from EBV immortalized B cell cultures (*B*) were tested for the presence of MERS CoV pseudovirus (London1/2012, abbreviated as LD1) neutralizing antibodies. LCA60 and LCA57 antibodies were tested for their ability to neutralize the homologous London1/2012 CoV (*C*) and bind to S protein (EMC/2012) (*D*). The LCA60 antibody was further tested against three infectious MERS CoV isolates (*E*) and several EMC MERS CoV MARMs (*F*).

3B11. In addition, the affinity of LCA60 binding to the RBD was ~500-fold higher compared with CD26.

LCA60 uses VH3 15\*01 and VL2 23\*01, has a 18-amino-acid HCDR3, and carries 17 amino acid substitutions in VH and 12 in VL. In silico analysis of the antibody sequence did not reveal any sequence motif that may impact expression or stability. A codon-optimized gene was synthesized and cloned into a double gene expression vector that was used to transfect the Lonza CHO-K1SV-GS-KO cell line. After a round of selection followed by subcloning, a high-producing research cell line, called LCA60.273.1, was iso-lated. This line showed high stability of expression and produced 4 5 g/L under fed-batch conditions in shake flasks. The whole process of isolating the antibody and producing the stable cell line was completed in less than 4 mo. This illustrates the feasibility of rapidly developing a human monoclonal neutralizing antibody in response to newly emerging life-threatening infections.

**Isolation of Viral Escape Mutants and Molecular Modeling of the RBD-LCA60 Complex.** LCA60 was tested against a panel of monoclonal antibody-resistant mutants (MARMs) that were previously selected using the phage-derived antibodies 1E9 (P547S), 1F8 (R542G), 3C12 (Y540H), and 3B12 (L506F/T512A), which were mapped to three different regions in the RBD (12). Of note, all these MARMs were efficiently neutralized by LCA60 (Fig. 1F). These results indicate that LCA60 binds to a

NoC



**Fig. 2.** LCA60 binds to S protein with high affinity. SPR sensograms showing the CoV S protein (EMC/2012) association and dissociation with the LCA57, LCA60, 3B11, and CD26 receptor. The antibodies and the receptor were immobilized on the sensor surface, followed by injection of S protein at the concentrations indicated in the figure. The line fitted to the experimental data and used to calculate the binding affinities is drawn in gray. KD values are reported in the table.

distinct site on the S protein. To better define the LCA60 epitope, we performed viral escape studies. Upon three rounds of selection with LCA60, two independent MARMs were isolated (Fig. 1*F*). The sequence of the S genes showed that both MARMs carried two identical mutations, V33A in the N-terminal region and E536A in the RBD (Fig. S1).

To map the LCA60 epitope, we used a combination of computational predictions and experimental validation. Computational docking predicts the atomic-level 3D structure of a multimolecular complex starting from the separated structures of the individual components, in this case RBD and LCA60. The experimental structure of MERS-CoV RBD was available (10, 11), whereas the structure of LCA60 was predicted by homology modeling according to the canonical structure method (15). The CDR loops of an antibody can adopt a limited and predictable set of conformations, dependent on length and presence of specific amino acids at key positions, with the exception of the HCDR3 loop, which can adopt variable and often flexible conformations due to the high variability in sequence and length (Fig. S2).

To cover a larger conformational space and to limit the impact of possible modeling inaccuracies, we generated multiple models of LCA60 and docked each of them independently to the MERS-CoV RBD structure using the RosettaDock program (16). The docking algorithm identified two equally plausible models of the LCA60 RBD complex (Fig. 3 A and B and Fig. S3), which we were able to discriminate according to their agreement with experimental data. In both models, the antibody interacts with RBD residues around K493, but only in model A does the antibody interact with residues in the 540 543 region. Introducing mutations in the latter region is expected to affect antibody binding if model A is correct, whereas these mutations

led by guest on April 21, 2020

NoC

Corti et al.

**PNA** 

should not affect binding if model B is correct. We thus produced 14 mutants of the S protein spanning different regions of the RBD, including the MARM mutations described above. LCA60 and LCA57 were then tested for their ability to bind to cells expressing full-length S mutants. Whereas LCA57 bound to all mutants, LCA60 did not bind T489A, K493A, and E565A and bound poorly to LCA60 MARM E536A (Table S1). These results allowed us to discard model A and select model B as the most consistent with experimental data. Importantly, the four positions that affected LCA60 binding (T489, K493, E565, and E536) are conserved in all natural isolates described to date, including the most recent isolates from the outbreak in South Korea (Fig. S4).

According to model B, the LCA60 footprint on the RBD partially overlaps with that of the CD26 receptor (Fig. 3 *C* and *D*), suggesting that LCA60 neutralizing activity results from interference with the RBD CD26 interaction. Results of surface plasmon resonance (SPR) cross-competition experiments showed that LCA60 and 3B11 compete for binding to RBD, whereas LCA57 did not compete with either LCA60 or 3B11. In addition, LCA60 and 3B11, but not LCA57, prevented the binding of CD26 to RBD (Fig. 4). This is in agreement with the structural model presented and indicates that LCA60 and 3B11 neutralize MERS-CoV by blocking binding to its cellular receptor.

**LCA60 Prophylactic and Postexposure Efficacy in MERS-CoV-Susceptible Mice.** The LCA60 produced in the CHO cell line was tested in BALB/c mice that were sensitized to MERS-CoV infection by transduction with adenoviral vectors expressing human CD26 (17). In this model, as a result of virus receptor expression on lung



**Fig. 3.** Computational models of MERS CoV RBD in complex with antibody LCA60. (*A* and *B*) Structures of the complex between mAb LCA60 (light green and orange cartoons) and RBD MERS (white surface) obtained by computational docking. S protein mutants that affect (red) or do not affect (blue) antibody binding are shown. Only model B is consistent with the muta genesis data. (C) Experimental structure of the complex between CD26 (light blue cartoon) and MERS RBD [Protein Data Bank (PDB) ID code 4KR0]. (*D*) Footprint of LCA60 antibody (light green) and CD26 (light blue) on the surface of MERS RBD (white). Residues in contact with both LCA60 and CD26 are shown in violet.



Fig. 4. LCA60 blocks MERS CoV RBD binding to its cognate cellular receptor CD26. Cross competition SPR assay was used to investigate the epitopes region; LCA57, LCA60, 3B11 antibodies and CD26 receptor (ligands) were first immobilized on different channels (vertical column) of the SPR chip. The spike protein was subsequently injected to form the complexes; finally, the three antibodies and the receptor (analytes) were injected one in each horizontal row. If a binding event is detected (red borders rectangle), the analyte has a different epitope from the ligand immobilized in the first step; if no binding is detected, ligand and analyte share overlapping epitopes.

cells, MERS-CoV gains entry to lungs and replicates as in human disease. However, replication and pathology are limited and the virus is cleared by day 7. hCD26-transduced mice were treated by i.p. injection of LCA60 at 1, 5, or 15 mg/kg either 1 d before (day 1) or 1 dafter intranasal infection (day +1) with EMC and London strains. The results showed that, in both prophylactic and post-exposure settings, LCA60 treatment led to a 2 4 log reduction in lung viral titers on day 3 with undetectable levels obtained in nearly all mice by day 5 (Fig. 5 A D). In the same model, LCA60 administered on day +1 by intranasal delivery resulted, when titers were measured at day 3, in a significant dose-dependent inhibition of viral titers that was still detectable when the antibody was dosed at 0.12 mg/kg (Fig. 5*E*).

We also used the mouse infection model to test whether the LCA60 MARM virus (carrying E536A and V33A mutations) might have an impaired fitness in vivo. The results showed that, as expected, LCA60 antibody was not effective prophylactically and that the mutant virus could replicate in CD26-transduced mice to levels comparable to the EMC parental virus (Fig. S5). We noted, however, that plaques of the LCA60 MARM were smaller in size compared with those of the original EMC strain.

The efficacy of LCA60 was also evaluated in a more stringent model of MERS-CoV infected IFN- $\alpha/\beta$  receptor-deficient mice (IFNAR-KO). Once transduced with human CD26, these mice developed a more profound clinical disease following MERS-CoV infection, as shown by a body weight loss, prolonged virus shedding, and gross changes in histology (17). Administration of LCA60 at 15 mg/kg i.p. 1 d after infection reduced lung viral titers by more than three logs in mice infected with both London and EMC strains on both day 3 and 5 (Fig. 6 *A* and *B*). In addition, LCA60 prevented body weight loss as well as perivascular and peribronchial lymphoid infiltration that in control mice further progressed to an interstitial pneumonia (Fig. 6 *C* and *D*). In the same model, LCA60 administered at 10 mg/kg on day +1 by intranasal delivery resulted in a significant inhibition of viral titers on day 2, and to a major extent on day 4, compared with control mice (Fig. 6F).

## Discussion

Clinical studies in patients with severe viral pneumonia caused by viral infections have shown that convalescent sera can have a therapeutic benefit. This has been demonstrated for infections including Spanish influenza pneumonia during 1918 1919 (18), SARS-CoV (19), H5N1 influenza A virus (20), and more recently severe H1N1 influenza A virus infection (21). However, the lack of availability of convalescent sera and the low antibody titers, like in the case of the patient described in this study, significantly limit the utility of this approach. Rapid isolation, purification, and production of specific human neutralizing monoclonal antibodies represent a safe, controlled, affordable, and unlimited source of the most potent antibodies present in convalescent sera.

Due to the continuous emergence of new zoonotic viruses, there is a need to develop rapid pathways for the identification and development of new treatments for prophylaxis, postexposure prophylaxis, and therapy. This study illustrates an example of this approach for MERS. In particular, we have shown that a human neutralizing antibody can be isolated, characterized, and produced in large amounts in cell lines compatible for human use in a short period of only a few months. The efficiency of the B-cell interrogation method used was instrumental in isolating a rare cell from a small blood sample obtained from an individual who had mounted only a modest neutralizing antibody response. The isolation of antibodies using phage libraries can also be rapid but bears some risks for their development. In particular, due to scrambled VH VL pairing, phage-derived antibodies carry a risk of autoreactivity and may not express well in mammalian cells, a fact that has significantly hampered their clinical development. Our data also show a rapid path for epitope mapping and mechanism of action studies using experimentally validated computational docking, viral escape mutant, and cross-competition assays.

Dow



**Fig. 5.** Passive transfer of LCA60 confers protection to mice prophylactically and therapeutically. We transferred 200 µL of diluted antibodies in PBS into Ad5 hDPP4 transduced BALB/c mice (6 wk, female) intraperitoneally 1 d before (A and C) or after (*B* and *D*) MERS CoV EMC/2012 (EMC) (A and *B*) or London1/2012 (LD1) (*C* and *D*) infection. Mice were then infected with  $1 \times 10^5$  pfu MERS CoV intranasally. Virus titers in the lungs were measured at the indicated time points. (*E*) Intranasally delivered LCA60 protected MERS CoV infected mice. We transferred 50 µL of diluted antibodies in PBS intranasally into Ad5 hDPP4 transduced BALB/c mice (6 wk, female) 1 d after infection with  $1 \times 10^5$  pfu of the EMC/2012 strain of MERS CoV. Virus titers in the lungs were measured on day 3 after in fection. Titers are expressed as pfu/g tissue. n = 3 4 mice per group per time point. \*P < 0.05 compared with hlgG1 group.

This study also takes advantage of recent developments of animal models for emerging respiratory viral infections based on transient expression of human receptors in wild-type or immunedeficient mice. In this model, our antibody showed high efficacy both in prophylaxis and therapy, with reduction of lung viral titers of 100 1,000-fold, as well as reduction of signs and pathology. There is increasing evidence that antibodies can be efficiently delivered through nebulization (22 24). It was interesting to find that when administered intranasally LCA60 was effective in both mouse models and even when tested at concentrations as low as 0.12 mg/kg. These results suggest that it would be important to evaluate the local delivery of an antibody by nebulization, a route of administration that offers considerable advantages over the parenteral route, such as lower dosage, higher antibody concentration in lung fluids, and ease of multiple administrations.

LCA60 binds to an epitope in the  $\hat{S}$  protein RBD with subnanomolar affinity and potently neutralizes MERS-CoV infection of multiple isolates. The epitope has partial overlap with the RBD CD26 receptor interface, and antibody binding prevents interaction of the viral RBD with its cellular receptor. Therefore, inhibition of virus receptor interactions is at least one major mechanism of LCA60 neutralization. Experimentally validated computational docking as well as generation of escape variants allowed identification of RBD residues critical for binding and neutralization. LCA60 has a novel epitope that is not affected by escape mutants generated by 3B11 and other phage-derived neutralizing antibodies.

The use of a single neutralizing antibody bears the risk of selecting escape mutants in vivo, a fact that has been demonstrated in vitro for LCA60 and the other described antibodies. The use of two antibodies against nonoverlapping sites may offer a solution to this problem. However, to date, all of the MERS-CoV neutralizing antibodies cross-compete, a finding that might not provide a rationale for combining these antibodies. Although recognizing that the full spectrum of MERS-CoV antigenic variation is still under study, it should be noted that the residues that are important for LCA60 binding are conserved in all MERS-CoV isolates, whereas 3B11 fails to recognize the London isolate due to the L506F mutation.

This work demonstrates the feasibility of using monoclonal antibodies isolated from memory B cells of survivors as a rapid response platform to protect the public health from emerging



Fig. 6. LCA60 protected MERS CoV infected IFNAR KO mice. We trans ferred 200 µL of diluted antibodies (15 mg/kg) in PBS into Ad5 hDPP4 transduced IFNAR KO mice (6 12 wk) intraperitoneally 1 d after MERS CoV infection. Mice were then infected with  $1 \times 10^5$  pfu MERS CoV London1/2012 strain (A) or EMC/2012 strain (B E) intranasally. Virus titers in the lungs were measured at the indicated time points. Additional groups of animals were monitored daily for morbidity, mortality, and body weight loss up to 14 d after infection (C). The histologic analysis was performed on animals killed on day 7 after infection (D and E). Mice were anesthetized and transcardially perfused with PBS followed by zinc formalin. Lungs were removed, fixed in zinc formalin, and paraffin embedded. Sections were stained with hema toxylin and eosin for histological analysis. (F) Intranasally delivered LCA60 protected MERS CoV infected mice. We transferred 50 µL of diluted anti body (10 mg/kg) in PBS intranasally into Ad5 hDPP4 transduced IFNAR KO mice (6 wk, female) 1 d after infection with  $1 \times 10^5$  pfu of the EMC/2012 strain of MERS CoV. Virus titers in the lungs were measured at the indicated time points. Titers are expressed as pfu/g tissue. n = 3 mice per group per time point. \*P < 0.05 compared with hlgG1 or PBS groups.

Downk

Corti et al.

ANG

viruses like SARS or MERS-CoVs, or others, which are likely to continue to emerge.

#### Methods

Detailed information about generation of anti MERS CoV monoclonal anti bodies, screening, in vitro characterization of antibodies, and testing in vivo can be found in *SI Methods*. All protocols in animal experiments were approved by the University of Iowa Institutional Animal Care and Use Committee.

- 1. Assiri A, et al.; KSA MERS-CoV Investigation Team (2013) Hospital outbreak of Middle East respiratory syndrome coronavirus. N Engl J Med 369(5):407–416.
- Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM (2012) Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 367(19):1814–1820.
- Ge X-Y, et al. (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503(7477):535–538.
- Memish ZA, et al. (2013) Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis 19(11):1819–1823.
- Haagmans BL, et al. (2014) Middle East respiratory syndrome coronavirus in dromedary camels: An outbreak investigation. *Lancet Infect Dis* 14(2):140–145.
- Kupferschmidt K (2015) Infectious diseases. MERS surges again, but pandemic jitters ease. Science 347(6228):1296–1297.
- Hofmann H, et al. (2004) S protein of severe acute respiratory syndrome-associated coronavirus mediates entry into hepatoma cell lines and is targeted by neutralizing antibodies in infected patients. J Virol 78(12):6134–6142.
- Gierer S, et al. (2013) The spike protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralizing antibodies. J Virol 87(10):5502–5511.
- Raj VS, et al. (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 495(7440):251–254.
- Lu G, et al. (2013) Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature 500(7461):227–231.
- 11. Wang N, et al. (2013) Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Res* 23(8):986–993.
- Tang X-C, et al. (2014) Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. *Proc Natl Acad Sci USA* 111(19): E2018–E2026.
- Ying T, et al. (2014) Exceptionally potent neutralization of Middle East respiratory syndrome coronavirus by human monoclonal antibodies. J Virol 88(14):7796–7805.
- Bermingham A, et al. (2012) Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill 17(40):20290.
- Pedotti M, Simonelli L, Livoti E, Varani L (2011) Computational docking of antibodyantigen complexes, opportunities and pitfalls illustrated by influenza hemagglutinin. Int J Mol Sci 12(1):226–251.
- Gray JJ, et al. (2003) Protein-protein docking with simultaneous optimization of rigidbody displacement and side-chain conformations. J Mol Biol 331(1):281–299.
- 17. Zhao J, et al. (2014) Rapid generation of a mouse model for Middle East respiratory syndrome. *Proc Natl Acad Sci USA* 111(13):4970–4975.

ACKNOWLEDGMENTS. R.S.B. is supported by National Institute of Allergy and Infectious Diseases (NIAID) Grants AI085524 and AI110700, and S.P. is supported by NIH Grants RO1 AI091322 and PO1 AI060699. M.Z. and R.G. are both supported by Public Health England. A.L. and L.V. are supported by Swiss National Science Foundation Grants 147662 and 138518, respec tively. A.L. is also supported by the Swiss Vaccine Research Institute. The Institute for Research in Biomedicine is supported by the Helmut Horten Foundation. We also acknowledge the contract support from NIAID HHSN272201000013I.

- Luke TC, Kilbane EM, Jackson JL, Hoffman SL (2006) Meta-analysis: Convalescent blood products for Spanish influenza pneumonia: A future H5N1 treatment? Ann Intern Med 145(8):599–609.
- 19. Cheng Y, et al. (2005) Use of convalescent plasma therapy in SARS patients in Hong Kong. *Eur J Clin Microbiol Infect Dis* 24(1):44–46.
- Zhou B, Zhong N, Guan Y (2007) Treatment with convalescent plasma for influenza A (H5N1) infection. N Engl J Med 357(14):1450–1451.
- Hung IF, et al. (2011) Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. *Clin Infect Dis* 52(4): 447–456.
- Respaud R, Vecellio L, Diot P, Heuze Vourc'h N (2014) Nebulization as a delivery method for mAbs in respiratory diseases. *Expert Opin Drug Deliv* 12(6):1027–1039.
- Leyva-Grado VH, Tan GS, Leon PE, Yondola M, Palese P (2015) Direct administration in the respiratory tract improves efficacy of broadly neutralizing anti-influenza monoclonal antibodies. *Antimicrob Agents Chemother* 59(7):4162–4172.
- Maillet A, et al. (2011) The airways, a novel route for delivering monoclonal antibodies to treat lung tumors. *Pharm Res* 28(9):2147–2156.
- Traggiai E, et al. (2004) An efficient method to make human monoclonal antibodies from memory B cells: Potent neutralization of SARS coronavirus. *Nat Med* 10(8): 871–875.
- Tiller T, et al. (2008) Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. J Immunol Methods 329(1-2):112–124.
- Temperton NJ, et al. (2005) Longitudinally profiling neutralizing antibody response to SARS coronavirus with pseudotypes. *Emerg Infect Dis* 11(3):411–416.
- Temperton NJ, et al. (2007) A sensitive retroviral pseudotype assay for influenza H5N1-neutralizing antibodies. *Influenza Other Respi Viruses* 1(3):105–112.
- Marcatili P, Rosi A, Tramontano A (2008) PIGS: Automatic prediction of antibody structures. *Bioinformatics* 24(17):1953–1954.
- Sircar A, Kim ET, Gray JJ (2009) RosettaAntibody: Antibody variable region homology modeling server. Nucleic Acids Res 37(Web Server issue):W474–W479.
- Simonelli L, et al. (2010) Rapid structural characterization of human antibody-antigen complexes through experimentally validated computational docking. J Mol Biol 396(5):1491–1507.
- 32. Agnihothram S, et al. (2014) Evaluation of serologic and antigenic relationships between middle eastern respiratory syndrome coronavirus and other coronaviruses to develop vaccine platforms for the rapid response to emerging coronaviruses. J Infect Dis 209(7):995–1006.