**INFECTIONOUS DISEASE**

**Recovery from the Middle East respiratory syndrome is associated with antibody and T cell responses**

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The Middle East respiratory syndrome coronavirus (MERS-CoV) causes a highly lethal pneumonia. MERS was recently identified as a candidate for vaccine development, but most efforts focus on antibody responses, which are often transient after CoV infections. CoV-specific T cells are generally long-lived, but the virus-specific T cell response has not been addressed in MERS patients. We obtained peripheral blood mononuclear cells and/or sera from 21 MERS survivors. We detected MERS-CoV–specific CD4+ and CD8+ T cell responses in all MERS survivors and demonstrated functionality by measuring cytokine expression after peptide stimulation. Neutralizing (PRNT50) antibody titers measured in vitro predicted serum protective ability in infected mice and correlated with CD4+ but not CD8+ T cell responses; patients with higher PRNT50 and CD4+ T cell responses had longer intensive care unit stays and prolonged virus shedding and required ventilation. Survivors with undetectable MERS-CoV–specific antibody responses mounted CD8+ T cell responses comparable with those of the whole cohort. There were no correlations between age, disease severity, comorbidities, and virus-specific CD8+ T cell responses. In conclusion, measurements of MERS-CoV–specific T cell responses may be useful for predicting prognosis, monitoring vaccine efficacy, and identifying MERS patients with mild disease in epidemiological studies and will complement virus-specific antibody measurements.

**INTRODUCTION**

The Middle East respiratory syndrome coronavirus (MERS-CoV), recently emerged from zoonotic sources, causes severe pneumonia in patients in the Middle East and in travelers from this region (1). As of 18 June 2017, 2029 cases with 704 deaths (34.7% case fatality rate) had been reported to the World Health Organization (WHO). MERS-CoV, like the coronavirus that caused the severe acute respiratory syndrome (SARS-CoV), has the potential to cause widespread outbreaks, as occurred in 2015 in South Korea (2). In this instance, a single patient with MERS entered the country, resulting in 186 secondary and tertiary cases and quarantining of about 16,000 individuals (2). Further, unlike SARS-CoV, MERS-CoV continues to be introduced from infected intermediates, most importantly, dromedary camels, to human populations (3). These observations indicate the need for understanding the human immune response to the virus to guide immunotherapy of severely ill patients and vaccine development and to develop additional tools for determining the prevalence of the infection.

Although clinical MERS has been well described, materials from autopsy specimens are available only for a single patient (4). Additionally, the MERS-CoV–specific immune response is not well characterized. In particular, it is known that virus-specific antibody responses can be identified in many but not all infected patients and are only transiently detected in some patients with pneumonia (5–7). In contrast, nothing is known about the T cell response to the virus, about how disease severity affects this response, and about the correlation of antivirus antibody with T cell responses. In SARS survivors, virus-specific antibody responses could no longer be detected at 6 years after infection, whereas T cell responses could be detected as long as 11 years after infection (8). Further, administration of convalescent sera is considered a potential therapeutic option (9), but levels of virus-specific antibody required for protection have not been established.

We report an analysis of the MERS-CoV–specific T cell responses in patients and show that CD8+ T cell responses can be detected in some patients with undetectable antibody responses. Our results also demonstrate the relationship between neutralizing antibody titers measured in vitro and protective levels in vivo. We additionally examined the relationship between virus-specific antibody and T cell responses and clinical parameters and identified T cell epitopes recognized in some patients. These results will be useful for identifying previously infected patients with low or nil antibody titers in epidemiological studies of the infection and for establishing guidelines for therapeutic use of convalescent sera in patients and will complement measurements of virus load in predicting patient outcomes.
RESULTS
We obtained peripheral blood mononuclear cells (PBMCs) and sera from 18 MERS survivors and sera from an additional 3 patients. Samples were obtained at 6 and 24 months after infection from 14, 4, and 3 patients in Riyadh, Jeddah, and Mekkah, respectively. Patient demographics and laboratory values including the cellular composition of PBMCs are shown in tables S1 and S2 (gating strategy is shown in fig. S1). Patients required hospitalization at about 7 days after the development of symptoms. These patients were tested serially and remained positive for MERS-CoV RNA for periods ranging from 7 to 45 days. Clinical severity ranged from asymptomatic/subclinical to severe, with most patients with severe disease requiring intensive care unit (ICU) care and ventilation. Of the 18 patients who provided PBMCs, 3 patients were asymptomatic, 6 patients had pneumonia, and 9 patients had severe pneumonia, requiring intubation and ventilation. Patients remained in the ICU for 2 to 74 days. All patients were discharged from the hospital by 174 days after admission. We also measured hepatic and renal function and found that, in general, renal and hepatic abnormalities were more common in patients with more severe respiratory disease (table S1).

Next, we analyzed sera for bulk MERS-CoV–specific antibodies, using enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA), and for neutralizing antibodies, using infectious MERS-CoV in microneutralization, and plaque reduction neutralization (PRNT<sub>50</sub>) assays (Table 1). Titers measured by the four different methods were generally consistent within individual patients. MERS-CoV–specific antibodies were undetectable or very low in three asymptomatic patients (patients 12 to 14) and in four patients with pneumonia or severe pneumonia (patients 3, 4, 7, and 21). This relationship between low antibody responses and less severe clinical disease was also observed in previous studies (5, 6, 10). To further assess the physiological contribution of the magnitude of the neutralizing antibody titers, we transferred 75 μl of serum from individual patients to mice sensitized for MERS-CoV infection using nonreplicating adenovirus vectors expressing the human receptor [human dipeptidyl peptidase 4 (Ad5–human DPP4 (hDPP4))] (11, 12). As shown in Fig. 1A and B and Table 1, PRNT<sub>50</sub> titers in the sera of recipient mice correlated well with titers in the human sera. Mouse PRNT<sub>50</sub> titers in the sera at the time of challenge correlated inversely with virus titers in the lungs, confirming the importance of neutralizing antibody assessed in vitro in virus clearance in vivo (Fig. 1C). These results also suggest that a PRNT<sub>50</sub> of >1:50 was required to reduce virus titers by 0.5 log in infected mice. Assuming that these numbers can be extrapolated to patients, transferring 75 μl of sera to a 25-g mouse is equivalent to transferring 210 ml of sera to a 70-kg patient (calculated on a per-kilogram basis), thereby providing a possible framework for its use in clinical settings.

To assess T cell responses, we synthesized a set of 20-mer peptides overlapping by 10 amino acids, encompassing the four MERS-CoV structural proteins (table S3), and used these peptides in a series of intracellular cytokine [interferon-γ (IFN-γ)] staining assays with PBMCs from healthy donors and MERS survivors. We used peptides instead of infectious virus for these assays because MERS-CoV has been shown to induce apoptosis in activated T cells, which, in these

<table>
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<th>IFA titer</th>
<th>Microneutralization titer</th>
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Fig. 1. Convalescent sera transfer protects mice from MERS-CoV infection. (A) Mice received 75 μl of patient serum intravenously 12 hours before MERS-CoV infection. One hour before infection, mice sera were collected and PRNT\textsubscript{50} assays were performed as described in Materials and Methods. HD, healthy donor; LOD, limit of detection. (B) Relationship between PRNT\textsubscript{50} in human sera and in mouse recipients of transferred sera. (C) To obtain virus titers, we homogenized lungs at day 3 after infection and titered on Vero 81 cells. Titers are expressed as PFU/g of tissue. \( n = 3 \) mice per group per time point. Right: Relationship between PRNT\textsubscript{50} in mouse sera and viral titers in mouse lungs. AT, adoptive transfer.

Assays, would be virus-specific CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells (13). Initially, we created four pools of peptides [S1, S2, N, and ME encompassing the N- and C-terminal portions of the spike (S) glycoprotein, the nulceocapsid (N) protein, and the transmembrane (M) and envelope (E) proteins, respectively]. No virus-specific CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell responses were detected in the four healthy donors after peptide stimulation (Fig. 2, A and C). In contrast, nearly all patients contained CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells that responded to all four peptide pools. Some patients mounted a 5- to 10-fold higher response to the peptide pools, especially to those encompassing the N (CD4\textsuperscript{+}) and M (CD8\textsuperscript{+}) proteins, compared with the average (Fig. 2, A to D). A summary of the total CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell responses to all four peptide pools is shown in Fig. 2E. For individual patients, the percentage of virus-specific CD4\textsuperscript{+} T cells was higher in patients with greater PRNT\textsubscript{50} neutralizing titers (green symbols in Fig. 2, B, E, and F), whereas there was no relationship between the percentage of CD8\textsuperscript{+} T cells responding to MERS-CoV peptides and the PRNT\textsubscript{50} response (Fig. 2, D to F). The virus-specific CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells were multifunctional, because a substantial fraction (CD4\textsuperscript{+} T cells) or most cells (CD8\textsuperscript{+} T cells) expressed two cytokines [IFN-γ and tumor necrosis factor (TNF)] (Fig. 3A and C). The CD4\textsuperscript{+} T cells were phenotypically effector memory (CD45RA\textsuperscript{−}CCR7\textsuperscript{−}) cells (Fig. 3B), whereas the virus-specific CD8\textsuperscript{+} T cell populations also included effector (CD45RA\textsuperscript{−}CCR7\textsuperscript{−}) cells (Fig. 3D). Thus, these cells are multifunctional and are expected to rapidly and efficiently respond to subsequent infection with MERS-CoV. Further, these data demonstrate that virus-specific CD8\textsuperscript{+} T cells were detectable in patients with undetectable antibody responses, suggesting that measurement of the CD8\textsuperscript{+} T cell response might be useful in longitudinal and prevalence studies.

Because one of our ultimate goals was to identify CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell epitopes that predict rapid recovery from primary infection and protection from subsequent challenge and would be useful for more precisely measuring T cell responses, we next used our peptide pools to identify individual target peptides. First, we performed human leukocyte antigen (HLA) typing for all 18 patients from whom we obtained PBMCs (table S4). Second, because DR2 and DR3 alleles are common in Saudi Arabian populations, recognized in 18 to 20% and 25 to 29% of patients, respectively (14, 15), we obtained mice transgenic for expression of these alleles and infected them with MERS-CoV. We harvested lung cells and stimulated them with individual MERS-CoV peptides (table S3). We identified five immunodominant DR2-restricted and DR3-restricted peptides using these mice (Fig. 4A) and then validated their identification in patients expressing DR2 or DR3 alleles (Fig. 4, B to D). Although nearly all of these peptides were recognized in patients, the responses to a few were especially prominent [e.g., S45 (DR2-restricted) and S106 (DR3-restricted); indicated in red boxes in Fig. 4] and might be useful for monitoring CD4\textsuperscript{+} T cell responses in future studies (Fig. 4, B and C). Summary data for patient DR2-restricted and DR3-restricted peptide pools are shown in Fig. 4D. For monitoring CD8\textsuperscript{+} T cell responses, we were unable to identify putative epitopes using commercially available humanized HLA-expressing mice. As an alternative approach, because the M and E proteins were prominent targets for the CD8\textsuperscript{+} T cell response in five patients (Fig. 2, C and D) and the M and E proteins are small (219 and 82 amino acids, respectively), we screened PBMCs using individual peptides (Fig. 4E). At least three M-specific peptides were recognized in these five patients. Two of these patients (patients 1 and 5) shared HLA-A11 and HLA-C*07, suggesting that peptide M19 is restricted by one of these alleles, whereas patients 8 and 18 both expressed HLA-B40/41/44 and HLA-C*07, suggesting that M13 is restricted by one of these molecules (Fig. 4E and fig. S3).

Next, we compared the levels of virus-specific antibody and T cell responses over several variables including patient age, sex, ventilation status, presence of comorbidities, length of viral shedding, and time in ICU. T cell and antibody responses tended to be lower (although not significantly different) at 24 compared with 6 months after infection (Fig. 5A), probably reflecting decay of the response with increased time after infection. Therefore, we confined our analyses to the 14 patients in the former group. There were no differences in the MERS-CoV–specific CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell and PRNT\textsubscript{50} responses between patients younger and older than 50 years (Fig. 5, B and C). Males and females mounted similar CD8\textsuperscript{+} T cell and PRNT\textsubscript{50} responses, but males exhibited greater CD4\textsuperscript{+} T cell responses (Fig. 5, B and C). We found no relationship between the height of the PRNT\textsubscript{50} and T cell responses and the presence
of comorbidities (Fig. 5, B and C). Patients with severe disease requiring ICU admission and ventilation had higher PRNT$_{50}$ and CD4$^+$ T cell but not CD8$^+$ T cell responses compared with asymptomatic patients (Fig. 5, B and C). Furthermore, patients with prolonged viral shedding had significantly higher antibody but not T cell responses compared with patients with more transient virus shedding (Fig. 5D). Virus-specific CD4$^+$ T cell and PRNT$_{50}$ correlated with length of stay in the ICU ($R^2 = 0.3005$ and $0.6243$, $P = 0.04$ and 0.0008, corrected Akaike information criterion (AICc) = 6.78 and 208.78, respectively), whereas CD8$^+$ T cell responses were negatively correlated, although this did not reach statistical significance ($R^2 = 0.2052$, $P = 0.10$) (Fig. 5E). No bivariate models showed improvement over the univariate models for PRNT$_{50}$ and CD8$^+$ T cells. In contrast, for CD4$^+$ T cells, the addition of viral shedding to length of stay in the ICU improved the model (AICc decreased from 6.78 to 3.20 in the bivariate model).

Holding viral shedding constant, a 10-day increase in length of ICU stay would result in a 0.15% increase ($P = 0.0010$) in CD4$^+$ T cells. Holding length of ICU stay constant, an increase in viral shedding by 10 days would result in a 0.31% decrease ($P = 0.0087$) in CD4$^+$ T cells.

**DISCUSSION**

Although there is no evidence that MERS-CoV has mutated to enhance virulence and transmissibility in humans since it was first identified in 2012 (16), it is also apparent that the virus continues to be introduced into human populations, most likely from camels (“primary cases”). A total of 140 new, mostly primary cases have been diagnosed in Saudi Arabia thus far in 2017 (as of 18 June 2017), showing that the disease continues to be a public health threat.
MERS was recently identified by the WHO and CEPI (Coalition for Epidemic Preparedness Innovations) as a prime candidate for vaccine development (17) because it poses a potential outbreak threat. Targeted vaccination of high-risk human populations or vaccination of the likely intermediate host, dromedary camels, is under consideration (18), but no vaccine is presently licensed for human use. Efforts to develop vaccines for use in humans have been hampered by a lack of understanding of protective immune responses. Here, we show that virus-specific T cell responses can be identified in all MERS survivors, even in those with mild or subclinical infection, in whom serological testing is often negative. We also identified specific HLA-restricted CD4+ and CD8+ T cell epitopes, which is the first step in ascertaining protective and possibly pathogenic responses to individual T cell epitopes in MERS patients.

Previous studies of MERS prevalence have been based on virus-specific antibody measurements (5). Our results, as well as those that show that antibody titers are often transient or low in magnitude (5, 6), suggest that the true incidence of the infection is much greater than is now recognized and that a more accurate estimation could be determined if T cell responses were also measured. This approach might also provide information about the true prevalence of the infection in Africa, where a high percentage of camels are seropositive for MERS-CoV antibodies but where only a few patients with detectable antibody and no patients with clinical disease have been identified (19). We observed that the virus-specific CD8+ T cell and antibody responses were not correlated, indicating that the CD8+ T cell response would be most useful in determining the true incidence of infection. Low or transient MERS-CoV–specific antibody responses also raised the concern that patients with mild disease would be susceptible to reinfection and the development of clinical disease on subsequent virus exposure. However, the presence of a virus-specific CD8+ T cell response in all survivors partly alleviates this concern, because memory CD8+ T cells, especially if they are at the site of infection (the respiratory tract), would be expected to initiate an early and protective host immune response (20).

Virus-specific PRNT50 and memory CD4+ T cell but not CD8+ T cell responses correlated with severe disease, using days in the ICU as a marker for severity. These findings suggest that higher virus-specific antibody responses in severely ill patients reflect prolonged exposure to virus antigen or higher viral load. Higher levels of MERS-CoV were detected in nasopharyngeal samples obtained from patients with more severe disease or death compared with survivors (21). Conversely, patients with more robust virus-specific CD8+ T cells may clear infectious virus and viral antigen more rapidly, resulting in lower CD4+ T cell and antibody responses. No information is yet available about T cell responses in patients who succumbed to the infection during the acute phase. However, on the basis of the magnitude of MERS-CoV–specific CD8+ T cell responses in survivors, their measurement might provide information relevant to prognosis while patients are still hospitalized: Patients with detectable virus-specific CD8+ T cell responses at earlier times after infection might be expected to have more favorable outcomes. Such information would complement assays measuring the kinetics of MERS-CoV shedding and clearance.

Fig. 3. Human PBMC-derived MERS-CoV–specific T cells are multifunctional. (A and C) PBMCs were stimulated with MERS-CoV structural protein–specific peptide pools. Frequency and percentage of cells expressing IFN-γ and TNF are shown. (B and D) PBMCs were stimulated with the N (B) or ME (D) peptide pools. CD4+ (B) or CD8+ (D) T cells were then analyzed for the indicated phenotypic markers.
Our observations and analyses will need to be confirmed with larger numbers of patients. We have thus far obtained PBMCs from 18 and sera from 21 previously infected individuals, which represent 2 to 3% of all reported MERS survivors in Saudi Arabia (www.moh.gov.sa/en/CCC/PressReleases/Pages/default.aspx). Longitudinal studies of previously infected patients will also be required to more precisely compare the longevity of the virus-specific T cell versus antibody responses. A potential limitation of our study is that MERS-CoV–specific T cell epitopes may cross-react with epitopes present in common upper respiratory tract infection–associated CoV, especially because some of the epitopes are present on conserved proteins, such as the N protein. Use of pools of immunogenic peptides mitigates this concern to a large extent, because several epitopes are immunogenic, and it is unlikely that most would be cross-reacting. None of these epitopes were recognized by T cells from any of the healthy donors that we tested. Also, similar levels of T cell responses were detected to epitopes on conserved proteins and on ones that are less conserved, such as the surface glycoprotein, especially the S1 part, which is highly divergent between different CoVs.

In summary, we found that all MERS survivors that we analyzed developed CD4+ and CD8+ T cell responses. We also defined a titer of neutralizing antibody that was able to effect virus clearance in an animal model and is predicted to be useful in clinical settings. Patients with mild or subclinical illness develop prominent virus-specific CD8+ T cell responses, which may provide an additional factor useful for predicting prognosis of hospitalized patients and will be useful in studies of transmission patterns and prevalence by identifying previously infected patients with undetectable antibody responses to MERS-CoV.

Fig. 4. Identification of MERS-CoV–specific T cell epitopes in mice and patients. (A) Single-cell suspensions were prepared from the lungs of MERS-CoV–infected DR2 and DR3 transgenic mice and stimulated with peptides for 5 to 6 hours in the presence of brefeldin A. (B to D) DR2- or DR3-restricted patient PBMCs were stimulated with peptide pools or individual peptides for 12 hours in the presence of brefeldin A. (E) Patient PBMCs were stimulated with the ME peptide pool or individual peptides for 12 hours in the presence of brefeldin A. Frequencies of MERS-CoV–specific T cells (determined by IFN-γ intracellular staining) are shown.
MATERIALS AND METHODS

Study design and participants

The overall objective of the study was to determine antibody and T cell responses in MERS survivors and correlate the results with clinical disease parameters. Four tertiary care hospitals in Saudi Arabia participated in this study, one from Riyadh, two from Jeddah, and one from Makkah. All hospitals had infection control departments, critical care units, and access to subspecialty consultant services. During the 2015 MERS outbreak at the National Guard Hospital in Riyadh, 94 patients were identified as infected using a real-time reverse

Fig. 5. Relationship between MERS-CoV–specific T cell and neutralizing antibody responses and disease variables and severity. (A) Relationship between T cell and PRNT₅₀ responses and time after infection when samples were obtained. Mon, months; n.s., not significant. (B and C) Relationship between T cell (B) and PRNT₅₀ (C) responses and comorbidity (comorbidity versus none), ventilator status, sex, and age. (D and E) Relationship between T cell and PRNT₅₀ responses and the duration of virus shedding (D) and length of ICU stay (E).
transcription quantitative polymerase chain reaction assay with specimens obtained by nasopharyngeal swab or bronchoalveolar lavage. Fifty-four patients survived and were contacted about providing blood samples for immune analyses. Fourteen patients agreed to participate. Similarly, 40 MERS patients were identified during the 2014 MERS outbreak in King Faisal Specialist Hospital and Research Center in Jeddah. Of the 29 survivors, 2 agreed to provide blood for further analysis. In King Fahad General Hospital in Jeddah, 61 cases were identified and 19 died. Of the 42 survivors, 2 agreed to participate. In Al Nour Specialist Hospital in Makkah, 30 cases were identified and 9 died. Three survivors provided blood for serological testing but not for T cell analyses. Control samples of PBMCs were obtained from four anonymous donors at the University of Iowa. In total, the patient cohort for this study consisted of 11 participants.

**Study approval**
The Institutional Review Boards of all the centers approved the study. Written informed consent was obtained from all study participants.

**Clinical and serological testing**
Patients’ medical records were reviewed for information on demographic characteristics, comorbidities, clinical presentation, ICU admission, radiographic findings, duration of viral shedding, hematological parameters, renal profile, hepatic profile, development of acute kidney injury, requirement for dialysis, treatments received, and outcome. Blood from the Riyadh and Jeddah patients was fractionated into sera and PBMCs. Anti–MERS-CoV antibody titers were initially quantified by ELISA and IFA performed in Jeddah and Riyadh as previously described (5, 22). The ELISA for MERS-CoV S-specific antibody was read as positive (>1.1), negative (<0.8), or borderline (0.8 and 1.1). Sera were then analyzed for neutralizing antibody titer as described below.

**Mice, virus, and cells**
Specific pathogen–free 6-week-old BALB/c mice were purchased from the National Cancer Institute and Charles River Laboratories International. HLA-DR2 (DRB1*1501) and HLA-DR3 (DRB1*0301) transgenic mice were produced as previously described (23, 24). Mice were maintained in the Animal Care Facility at the University of Iowa. All protocols were approved by the University of Iowa Institutional Animal Care and Use Committee. The EMC/2012 strain of MERS-CoV (passage 8, designated MERS-CoV) was provided by B. Haagmans and R. Fouchier (Erasmus Medical Center). All work with infectious MERS-CoV was conducted in the University of Iowa biosafety level 3 (BSL3) laboratory.

**Antibody treatment and MERS-CoV infection of mice**
Because mice do not express a functional receptor for MERS-CoV, 6-week-old female BALB/c mice were lightly anesthetized with isoflurane and transduced intranasally with 2.5 × 10^6 plaque-forming units (PFU) of Ad5-hDPP4 in 75 µl of Dulbecco’s modified Eagle’s medium (DMEM) as described (11). Five days after transduction, mice were infected intranasally with MERS-CoV (1 × 10^5 PFU) in a total volume of 50 µl of DMEM. Mice were monitored daily for morbidity (weight loss) and mortality. All work with MERS-CoV was conducted in the University of Iowa BSL3 laboratory. Mice were injected with 75 µl of human serum intravenously 12 hours before MERS-CoV infection. Control mice were given an equal volume of healthy donor serum.

**Virus titers**
To obtain virus titers, we harvested lungs from subgroups of three animals at the indicated time points (see Results) and homogenized into 3 ml of phosphate-buffered saline, using a manual homogenizer. Lung homogenates were aliquoted and kept at −80°C. Virus was titered on Vero 81 cells (11). Viral titers are expressed as PFU/g of tissue for MERS-CoV.

**MERS-CoV microneutralization assays**
Serial twofold dilutions of human sera were prepared, and equal volumes of MERS-CoV (EMC/2012) and sera were combined and incubated for 1 hour at room temperature. The mixture was then added in quadruplicate to Vero 81 cells. The neutralization titer is the reciprocal of the highest serum dilution that neutralized the infectivity of 100 TCID₅₀ (median tissue culture infectious dose) of virus, read as the absence of cytopathic effect in the cells on day 4 after infection.

**MERS-CoV PRNT₅₀ assay**
Serum samples were serially diluted in DMEM and mixed with an equal volume of MERS-CoV (EMC/2012) containing 80 PFU. After incubation at 37°C for 1 hour, aliquots were added to cultures of Vero 81 cells in 48-well plates and incubated at 37°C in 5% CO₂ for 1 hour. Virus titers (PRNT₅₀) were determined as described (25).

**Preparation of cells from mouse lungs**
Mice were sacrificed at day 8 after infection. Lungs were removed, cut into small pieces, and digested in Hanks’ balanced salt solution buffer containing 2% fetal calf serum, 25 mM Hepes, collagenase (1 mg/ml) (Roche), and deoxyribonuclease (0.1 mg/ml) (Roche) for 30 min at 37°C. Tissues were dispersed using a 70-µm cell strainer, and single-cell suspensions were prepared. Live cells were enumerated by 0.2% trypsin blue exclusion. Cells were stimulated with peptides for intracellular cytokine expression as described previously (26).

**Flow cytometry**
The following anti-human monoclonal antibodies were used: CD3 (HI3t3a), CD4 (RPA-T4), CD8 (SK1), CD14 (M5E2), CD19 (SJ25C1), CD56 (5.1H11), T cell receptor γδ (B1), IFN-γ (B27), TNF (MAb11), CD45RA (HI100), CD27 (M-T271), and CCR7 (G043H7); all antibodies were from BD Biosciences, eBioscience, or BioLegend. Fc receptor blocking solution was obtained from BioLegend.

PBMCs were prepared from blood samples at the Riyadh and Jeddah sites using Lympholyte-H (Cedarlane) by following the product instruction. Cells were stored in liquid nitrogen before and during shipping to the University of Iowa where the cells were further analyzed. For surface staining, 10⁵ to 10⁶ cells were blocked with Fc receptor blocking solution, labeled with LIVE/DEAD staining dye (Thermo Fisher), and then stained with the indicated antibodies at 4°C. For intracellular cytokine staining, 10⁵ to 10⁶ cells per well were cultured in 96-well round-bottom plates at 37°C for 12 hours in the presence of 2 µM peptide (GenScript) and brefeldin A (BD Biosciences). Cells were then labeled for cell surface markers, fixed/permeabilized with Cytofix/Cytoperm Solution (BD Biosciences), and labeled with anti-intracellular cytokine/protein antibodies. All flow cytometry data were acquired on a BD FACSVerse and analyzed using FlowJo software (Tree Star Inc.).

**Statistical analysis**
Mann-Whitney test was used for initial analyses comparing the differences between groups, with P < 0.05 being considered statistically
Supplementary materials

References and Notes


Acknowledgments: We thank K. Kedziorska and Z. Wang for helpful discussions and A. Fehr, J. Zheng, R. Channanapavar, and D. L. Wheeler for critical review of the manuscript. We thank
all the patients who took part in this study. **Funding:** This study was funded by grants from the NIH (PO1 060699), 2015 Thousand Talents Plan Award of China, the Municipal Healthcare Joint-Innovation Major Project of Guangzhou (201604020011), and the Division of Intramural Research of National Institute of Allergy and Infectious Diseases, NIH. **Author contributions:** Jingxian Z., A.N.A., S.P., and Jincun Z. conceived the study. W.A.A., S.A.B., L.A.L., M.G.A., and M.M.A.G. were site investigators. I.K., S.M.A.J., A.M.D., and M.B. acquired and validated the data. Jingxian Z., Jincun Z., L.V., A.A.B., and A.M.N. executed the experiments. Jingxian Z., Jincun Z., S.P., L.V., and K.S. analyzed the data. A.M. provided the reagents. P.T.E. and C.W. performed the statistical analyses. Jingxian Z., A.N.A., S.P., Jincun Z., and P.T.E. wrote the manuscript. **Competing interests:** The authors declare that they have no competing interests.

Submitted 27 April 2017
Accepted 26 June 2017
Published 4 August 2017
10.1126/sciimmunol.aan5393

Recovery from the Middle East respiratory syndrome is associated with antibody and T cell responses


DOI: 10.1126/sciimmunol.aan5393

Breaking the camel virus’s back

Middle East respiratory syndrome coronavirus (MERS-CoV) causes a potentially lethal zoonotic pneumonia that can transfer between individuals after initial exposure to an infected camel. Now, Zhao et al. dig deeper into the immune response in MERS-CoV survivors. They found that neutralizing antibody titers could predict protection in an animal model but that antibody levels were often transient. Moreover, both CD4+ and CD8+ T cells were induced after MERS-CoV infection, and these cells could be detected even in the absence of virus-specific antibody. These data suggest that T cells may be useful in detecting mild or subclinical infection and that epitopes recognized by these T cells may form the basis for future vaccine design and immunotherapy.