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Passive Immunotherapy With Dromedary Immune Serum In An Experimental Animal Model For MERS Coronavirus Infection.

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Running title: Treatment of MERS with MERS convalescent camel sera

Abstract: The Middle East Respiratory Syndrome (MERS) is a highly lethal pulmonary infection. Sera from MERS convalescent patients may provide some benefit but is not readily available. In contrast, nearly all camels in the Middle East were previously infected with MERS-CoV. Here, we show that sera obtained from MERS immune camels augment the kinetics of MERS-CoV clearance, and reduce the severity of pathological changes in infected lungs, with efficacy proportional to MERS-CoV neutralizing serum antibody titers.

Importance.

The Middle East Respiratory Syndrome, caused by a coronavirus, is highly lethal with a case-fatality rate of 35-40%. No specific therapy is available and care is generally supportive. One promising approach is passive administration of sera from MERS convalescent humans or other animals to exposed or infected patients. The vast majority if not all camels in the Middle East were previously infected with MERS-CoV and some contain high titers of antibody to the virus. Here, we show that this antibody is protective if delivered either prophylactically or therapeutically to mice infected with MERS-CoV, indicating that this may be a useful intervention in infected patients.

Introduction:

A decade after the emergence of the Severe Acute Respiratory Syndrome (SARS), a novel beta coronavirus was isolated from a patient with a fatal viral pneumonia in Saudi Arabia in 2012 (1). The disease is now designated as the Middle East Respiratory Syndrome (MERS) and the causative virus is MERS coronavirus (MERS-CoV). So far (as of 7 February 2015), 971 confirmed cases, 356 of them fatal, have been reported to the World Health Organization (http://www.who.int/csr/disease/coronavirus_infections/mers-5-february-2015.pdf?ua=1). Primary human cases have been reported from a number of countries in the Arabian peninsula and the Middle Eastern region but travel-associated cases or limited human-to-human transmission from such cases have been reported from other countries in Europe, Africa and Asia. While clusters of human cases with limited human-to-human transmission within health care facilities or families have been reported (2), index cases in the transmission-chains remain of presumed zoonotic origin.

MERS-CoV-like viruses are widespread in dromedary camels with sero-epidemiological studies indicating sero-prevalence of >90% in adult animals (3). Viruses isolated from dromedaries are genetically and phenotypically closely related to virus isolated from humans and retain the capacity to infect *ex vivo* cultures of the human airways (4). Other domestic livestock in affected areas, including cattle, goats, sheep and equids, have no evidence of MERS-CoV infection. There is no convincing evidence of MERS-CoV in bats although a genetically related virus, albeit with a divergent spike protein, has been reported in *Neoromicia capensis* bats from Africa (5).

Infection in dromedaries has been reported to precede human infection in a few instances (6). Given the ubiquitous nature of infection in dromedaries, human exposure to MERS-CoV must be common; however, human disease remains rare (7). Furthermore, MERS-CoV remains endemic in dromedaries in East and North Africa (3) although locally acquired human cases have not been reported in countries in these regions. It is unclear whether this represents a lack of recognition or a true absence of disease.

Thus, while dromedaries are recognized as a natural host of MERS-CoV, the modes of transmission to humans remain unclear.

The apparent case-fatality of MERS appears to be high (approx. 37%) with age and underlying disease conditions including diabetes, respiratory or cardiovascular diseases or immune-compromised status being risk factors (8). When human case clusters have been intensively investigated, it is apparent that milder cases are not uncommon and that such cases are probably undiagnosed in the general population (2). Thus the overall severity of MERS may be milder than reflected from hitherto diagnosed cases. The repeated emergence of clusters of human-to-human MERS transmission is reminiscent of the emergence of SARS in late 2002, when clusters of human cases from the animal reservoir emerged and then went extinct, until the virus finally adapted to acquire capacity for sustained human-to-human transmission. Virus then spread globally to infect greater than 8000 persons in >28 countries or territories (reviewed in (9)). Within the past 200 years, other animal coronaviruses have adapted to humans and have spread globally – viz human coronaviruses 229E and OC43 (10). Thus, zoonotic MERS-CoV remains a concern for global public health.

So far, no clinically effective therapeutics have been identified. Some drugs, including some licensed for human use in other clinical indications, have activity *in vitro* but it is unclear whether their pharmacology and toxicity would allow therapeutic efficacy in humans (11, 12). Passive immunotherapy using convalescent human plasma is being considered for a number of emerging infectious diseases (e.g. MERS, influenza, Ebola) (11, 13). It was used for treatment of SARS with potentially promising results, although in the absence of controlled clinical trials, the results remain inconclusive (13, 14). The limited number of MERS-survived patients who are fit to donate plasma together with low convalescent antibody titers have constrained its use in MERS. On the other hand, dromedaries in the Middle East and in parts of Africa have high sero-prevalence and many of them have very high neutralizing antibody titers, presumably maintained through repeated re-infections. They have unusual single chain

immunoglobulins that may have theoretical advantages for passive immunotherapy (15). In this study, we have used a mouse model sensitized to MERS-CoV though transduction of the human DPP4 receptor (16) to investigate the prophylactic and therapeutic efficacy of dromedary serum containing high titer neutralizing antibodies to MERS-CoV in reducing viral load, weight loss and lung pathology.

Assay for neutralizing antibody in dromedary camels. Serum samples were collected from dromedaries in Egypt (collected 2013) and Australia (collected 2014) as previously described (17, 18). Samples from Egypt contained antibody to MERS-CoV while sera from Australian camels served as negative controls since all dromedary camels from Australia tested thus far are negative for MERS-CoV-specific antibody. Multiple aliquots were prepared to avoid repeated freezing and thawing of the sera. Antibody titers to MERS-CoV were determined using a well characterized and validated lentivirus-based pseudoparticle neutralization test as previously described (18). In this assay, pseudoparticles expressing the MERS-CoV spike protein of the EMC/2012 virus strain are exposed to antibodies under investigation and neutralizing titers are calculated. A panel of sera was selected to represent a range of MERS-CoV antibody titers found in dromedaries sampled in the field (Table 1). Sera from Egypt (Nos 1-6) had reciprocal antibody titers of 1:160-1:1280 while sera from Australia (Nos 21, 23) were, as expected, anti-MERS-CoV antibody negative.

Protective efficacy in MERS-CoV-infected mice. Mice are resistant to infection with MERS-CoV but can be rendered susceptible if the human dipeptidyl peptidase receptor (hDPP4) is supplied exogenously. We showed previously that transduction with an adenovirus vector expressing hDPP4 sensitizes mice to subsequent challenge with the MERS-CoV strain EMC/2012 (16). Virus is cleared within 7-10 days, with inflammatory cell infiltration apparent on histological examination of the lungs. Six to ten week old immunocompetent mice lose minimal amounts of weight and show no signs of clinical disease. However, mice lacking expression of the type 1 interferon receptor ($IFNAR^{-/-}$ mice) are more susceptible to the infection, showing weight loss and more extensive inflammatory cell infiltration and edema on

pathological examination of lung tissue. Initially, we assessed whether antibody from MERS immune camels was protective if 200 µL were delivered to immunocompetent BALB/c mice 1 day prior to infection with 1 x 10^5 PFU MERS-CoV (all protocols were approved by the University of Iowa Animal Care and Utilization Committee). Virus titers were measured at day 3 post infection (p.i.), since we showed previously that this is a useful time for assessing efficacy of anti-MERS-CoV prophylactic treatment (16). As shown in Figure 1A, virus was partially or completely cleared by day 3 p.i. from infected mice, with the protective ability of each sera proportional to the neutralizing titer measured *in vitro*. Further testing showed that sera from camel 2 could be diluted four fold without loss of ability to effect virus clearance by day 3 p.i. (Figure 1B).

If camel sera were to be useful in patients, it would need to be delivered therapeutically. Since Ad5-hDPP4-transduced mice lacking expression of the IFNα/β receptor ($IFNAR^{-/-}$) are more susceptible to MERS-CoV than immunocompetent mice, we treated $IFNAR^{-/-}$ mice with sera from camel 2 at 24 hours after infection. Under these conditions, treatment with undiluted sera accelerated the kinetics of virus clearance, with MERS-CoV nearly undetectable by five days after challenge (Figure 1C). Unlike BALB/c mice, $IFNAR^{-/-}$ mice exhibit weight loss after MERS-CoV infection. Treated, as opposed to untreated MERS-CoV-infected mice exhibited less weight loss (Figure 1D). Consistent with these results, decreased amounts of perivascular and peribronchial inflammatory cell infiltration, hemorrhage and edema were apparent on histological examination of infected lungs when treated and untreated infected $IFNAR^{-/-}$ mice were compared (Figure 2).

Implications for MERS-CoV-infected patients. In this study, we show that prophylactic or therapeutic treatment with high titer MERS immune camel sera is able to diminish weight loss and lung histological changes and effect virus clearance in mice infected with MERS-CoV EMC/2012. At this point, it is not possible to determine the effects of any treatment on severe clinical disease. Nonhuman primates and rabbits can be infected with MERS-CoV but none, except perhaps marmosets, develop severe clinical

disease (19, 20). Marmosets variably develop severe disease and are not readily available. Transgenic mice expressing hDPP4 may provide an alternative approach, but these mice either remain asymptomatic (unpublished results) or develop clinical disease, the significance of which is confounded by the concomitant development of severe encephalitis ((21) and our unpublished data). Our experiments were performed with a single strain of MERS-CoV, but it is likely that other strains will be neutralized as well. While evidence for evolution of human MERS-CoV has been reported, virtually no mutations are detected in the receptor binding domain, the most important site of neutralization. The most consistent change, at amino acid 1020 of the spike protein (22), is in the putative fusion domain, but even this change may reflect tissue culture adaptation. Comparison of cross-neutralizing antibody titers of MERS-CoV from Saudi Arabia belonging phylogenetically to clade B and genetically diverse viruses from Egypt demonstrated no reduction in neutralizing potency of dromedary camel sera against the latter (17).

While changes in clinical disease and blood laboratory parameters have been well documented in MERS in patients, little is known about changes in virus load and tissue damage as disease progresses because human specimens are not available. However, based on information accrued from the 2002-2003 epidemic of the Severe Acute Respiratory Syndrome, it is likely that a favorable outcome will occur when virus clearance occurs rapidly, providing time for the development of a protective antibody and T cell responses which then definitively clear the virus. Camel sera clearly reduce virus titers, when delivered either prophylactically or therapeutically. Use of camel sera has several advantages, including ready availability in the Arabian peninsula, the site of all initial infection thus far, and the presence of high titers of MER-CoV-specific antibodies. High anti-virus antibody titers are believed to reflect repeated infection of camels, perhaps during the birthing season (23). Based on a study of experimentally infected camels (24), MERS-CoV causes a moderate rhinitis with high virus loads detected in the nasal secretions, which would facilitate repeated infections. Camel antibodies have certain other advantages including a

long CDR3 region, which enhances the ability to recognize structures not detected by conventional antibodies, increased stability compared to conventional antibodies and relative ease of high level production (15). Heterologous (e.g. equine) antibodies have been successfully used in the past for passive immunotherapy or immunoprophylaxis of diseases such as rabies, tetanus and snake bites but such therapy carries a potential, though low risk from hypersensitivity to parenteral injection of protein from a different species. In the longer term, recombinant camelid antibodies can be expressed and multimerized, which results in enhanced avidity and humanized to reduce the risk of hypersensitivity (25).

In summary, our results provide proof of concept that sera from MERS-CoV immune dromedary camels are potentially useful in treatment of patients with MERS. Efficacy is most likely if delivered early in the course of illness (11). Furthermore, camels immunized with MERS-CoV can serve as the initial source for developing recombinant, humanized single stranded antibodies, as an additional tool for prophylactic or therapeutic treatment of exposed or infected patients, respectively.

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Figure Legends

Figure 1. Enhanced kinetics of MERS-CoV clearance after treatment with convalescent camel sera. Mice were sensitized to infection with MERS-CoV by transduction with Ad5-hDPP4. Five days later, mice were then challenged intranasally with 1 x 10^5 PFU of MERS-CoV EMC/2012, kindly provided by Dr. Bart Haagmans, Erasmus Medical Center, The Netherlands. All work with infectious MERS-CoV was performed in a Biosafety Level 3 (BSL3) laboratory. A. 200 µl of camel serum was transferred intraperitoneally into Ad5-hDPP4 transduced 6-8 wk old female BALB/c mice 24 hours before MERS-CoV

infection. Virus titers in the lungs were measured at day 3 post infection. Titers are expressed as PFU/g tissue. n= 3 mice/group/time point. *P values of <0.05 as compared to the No Treatment group. B. 200 µl of camel serum #2 diluted in PBS was transferred into Ad5-hDPP4 transduced old BALB/c mice as in (A). Virus titers were measured at day 3 p.i. n= 3 mice/group/time point. C. 200 µl of camel sera #2 diluted in PBS were transferred intraperitoneally into 6-10 week Ad5-hDPP4 transduced IFNAR$^{-/-}$ mice one day after intranasal infection with 1 x 10^5 PFU MERS-CoV. Titers were measured at days 3 and 5 p.i. n = 3 mice/group/time point. D. 200 µl of camel sera #2 were transferred intraperitoneally into Ad5-hDPP4 transduced IFNAR$^{-/-}$ mice one day after MERS-CoV infection. Mice were monitored daily for mortality (there was none) and weight loss. n= 5 mice per group.

Figure 2: Decreased severity of histological changes in MERS-CoV infected mice after therapeutic treatment with convalescent camel sera. IFNAR$^{-/-}$ mice were transduced with Ad5-hDPP4, not treated or treated with 200 µl camel sera #2 one day before infection with MERS-CoV EMC/2012 (1 x 10^5 PFU). At 7 days post infection, mice were anesthetized and perfused via the right ventricle with PBS followed by zinc formalin. Lungs were removed, fixed in zinc formalin, and paraffin embedded. Sections were stained with hematoxylin and eosin for histological analysis. (Left): Lungs from uninfected mice. (Middle and right): Lungs from MERS-CoV-infected mice at 7 days post challenge and either untreated mice (middle). or treated with 200 µl of camel sera #2 intraperitoneally (right). Multifocal peribronchial and perivascular infiltration, hemorrhage and edema (*) are observed in untreated samples (middle), but only minimal cellular infiltration is detected in mice that received MERS-CoV-specific camel antibody (right). Original magnification was 10x (upper panels) or 40x (lower panels).

207 **Table: MERS-CoV neutralizing antibody titers of dromedary camel sera assayed in a MERS-CoV spike**
208 **pseudoparticle microneutralization test**

Camel sera ID	Geographic origin	Reciprocal neutralizing antibody titer
1	Egypt	1:640
2	Egypt	1:1280
3	Egypt	1:640
4	Egypt	1:640
5	Egypt	1:160
6	Egypt	1:320
21	Australia	<1:10
23	Australia	<1:10

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