INFECTIOUS DISEASE

Human polyclonal immunoglobulin G from transchromosomic bovines inhibits MERS-CoV in vivo

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As of 13 November 2015, 1618 laboratory-confirmed human cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection, including 579 deaths, had been reported to the World Health Organization. No specific preventive or therapeutic agent of proven value against MERS-CoV is currently available. Public Health England and the International Severe Acute Respiratory and Emerging Infection Consortium identified passive immunotherapy with neutralizing antibodies as a treatment approach that warrants priority study. Two experimental MERS-CoV vaccines were used to vaccinate two groups of transchromosomic (Tc) bovines that were genetically modified to produce large quantities of fully human polyclonal immunoglobulin G (IgG) antibodies. Vaccination with a clade A γ-irradiated whole killed virion vaccine (Jordan strain) or a clade B spike protein nanoparticle vaccine (Al-Hasa strain) resulted in Tc bovine sera with high enzyme-linked immunosorbent assay (ELISA) and neutralizing antibody titers in vitro. Two purified Tc bovine human IgG immunoglobulins (Tc hIgG), SAB-300 (produced after Jordan strain vaccination) and SAB-301 (produced after Al-Hasa strain vaccination), also had high ELISA and neutralizing antibody titers without antibody-dependent enhancement in vitro. SAB-301 was selected for in vivo and preclinical studies. Administration of single doses of SAB-301 12 hours before or 24 and 48 hours after MERS-CoV infection (Erasmus Medical Center 2012 strain) of Ad5-hDPP4 receptor–transduced mice rapidly resulted in viral lung titers near or below the limit of detection. Tc bovines, combined with the ability to quickly produce Tc hIgG and develop in vitro assays and animal model(s), potentially offer a platform to rapidly produce a therapeutic to prevent and/or treat MERS-CoV infection and/or other emerging infectious diseases.

INTRODUCTION

The World Health Organization has identified Middle East respiratory syndrome coronavirus (MERS-CoV) infection as a "threat to global health" (*1*). As of 13 November 2015, 1618 laboratory-confirmed human cases of MERS-CoV infection, including 579 deaths, have been reported (*2*). MERS-CoV infection has been transmitted person to person, primarily in hospital settings (*3*). Most cases originated in Saudi Arabia, with travelers exporting cases to other countries, such as South Korea, which resulted in 186 cases of MERS-CoV infection and 36 deaths (*4*). MERS-CoV infection severity ranges from asymptomatic infection to severe respiratory distress and organ failure (*3*, *5*, *6*). The genome sequence of MERS-CoV has similarities to bat coronaviruses (BtCoV HKU4 and HKU5). The presence of a MERS-CoV RNA in an Egyptian tomb bat (*Taphozous perforatus*) suggests that bats may be the original zoonotic host of MERS-CoV (*7*, *8*). Blood samples from dromedary camels contain antibodies to MERS-CoV, and nasal swabs revealed that camels harbor the virus (*9*–*15*). Experimental infections of camels with MERS-CoV cause a transient mild-to-moderate disease (*16*).

Coronaviruses attach to host cells via their spike (S) glycoprotein. The S glycoprotein consists of an N-terminal S1 domain that contains the receptor-binding domain (RBD) and an S2 domain that mediates virus-cell fusion. The MERS-CoV receptor dipeptidyl peptidase 4 (DPP4) (*17*) is a multifunctional transmembrane endopeptidase that cleaves a protein regulator of insulin and other peptide hormones (*17*–*19*). The MERS-CoV RBD has been cocrystallized with the human DPP4 (hDPP4) protein (*20*), and antibodies to the RBD neutralize coronaviruses in vitro (*21*–*26*). In a mouse model of severe acute respiratory syndrome coronavirus (SARS-CoV) infection, vaccine-induced and passively transferred neutralizing antibodies have proven to be effective (*27*). A study comparing SARS-CoV vaccines revealed vaccine-induced immunopathology (*28*). One possible explanation for this immunopathology is antibody-dependent enhancement (ADE) of infection. Although ADE of infection has been reported for in vitro SARS-CoV experiments, its clinical significance is unknown (*9*–*15*).

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Human convalescent plasma, human-derived hyperimmune immunoglobulins (Igs), and monoclonal antibodies can prevent and treat many viral infections with less potential for severe reactions seen with animal-derived hyperimmune Igs (*29*, *30*). Public Health England identified passive immunotherapy as a treatment approach for MERS-CoV that warrants priority study (*31*). However, the ability to rapidly produce sufficient quantities of monoclonal antibodies, convalescent plasma, and/or hyperimmune human-derived Igs can be hampered by technical, logistical, and economic factors.

A potential source of human antibodies for passive immunotherapy is from transchromosomic (Tc) bovines that endogenously produce fully human polyclonal antibodies and mount a robust antibody immune response after vaccination (*32*–*38*). Tc bovines have genome knockouts of bovine Ig genes and carry a human artificial chromosome (HAC) composed of the entire germline loci of human Ig heavy chain and κ light chain. The Tc bovines produce fully human polyclonal IgG antibodies (up to 15 g/liter) and can be bioengineered to have an IgG1 subclass bias (*32*). Human IgG (hIgG) is then purified from pooled convalescent plasma obtained from vaccinated Tc bovines to produce 150 to 600 g of Tc hIgG per animal per month.

A collaboration of virologists, immunologists, and clinicians was formed to produce and evaluate an anti–MERS-CoV Tc hIgG. The authors on this study (see Author contributions) characterized a new strain of MERS-CoV (Jordan-N3/2012) and made it available for research (*39*). Two experimental MERS-CoV vaccines were produced and administered to two groups of Tc bovines. The first vaccine was a whole killed Jordan strain (clade A) virion vaccine (WKVV), and the second vaccine was an Al-Hasa strain (clade B) spike protein nanoparticle vaccine (SPNV). Two separate Tc hIgG were produced from large pools of plasma collected from two Tc bovine groups vaccinated with either WKVV (termed SAB-300) or SPNV (termed SAB-301). SAB-300 and SAB-301 antibodies were then evaluated by enzyme-linked immunosorbent assay (ELISA), in vitro neutralization, ADE assays, and in vivo studies. We now report on these results and plan for further preclinical evaluation and development of an anti–MERS-CoV therapeutic agent.

RESULTS

Vaccination and plasma collection

Two groups of Tc bovines bioengineered to have an IgG1 subclass proportion bias were vaccinated on five occasions (V1 to V5) every 21 to 28 days with WKVV (bovine #2244, #2252, and #2254) or with SPNV (bovine #2178 and #2183). High volumes of plasma were successfully collected by plasmapheresis following vaccinations V2 to V5 (Fig. 1A).

ELISA and IgG subclass proportions

Comparison of serum MERS-CoV ELISA antibody titers from vaccinations V2 to V5 of Tc bovines with WKVV or SPNV to negative control serum obtained before the first vaccination (V1) indicated a significant difference of $P \leq 0.0003$ between V2 to V5 versus pre–vaccination 1 day 0 (V1D0) serum [Dunnett's test following analysis of variance (ANOVA); Fig. 1B and table S1, A and B]. High SAB-300 and SAB-301 ELISA antibody titers (Fig. 1C and table S1, C and D) were found in V2 to V5 serum compared to that observed in negative control ($P < 0.0001$, Dunnett's test following ANOVA). SAB-300, SAB-301, and human-derived control intravenous immune globulin (IVIG) were found to have all human IgG subclasses, with a proportion of human IgG1 of 99%, 93%, and 67%, respectively (Table 1).

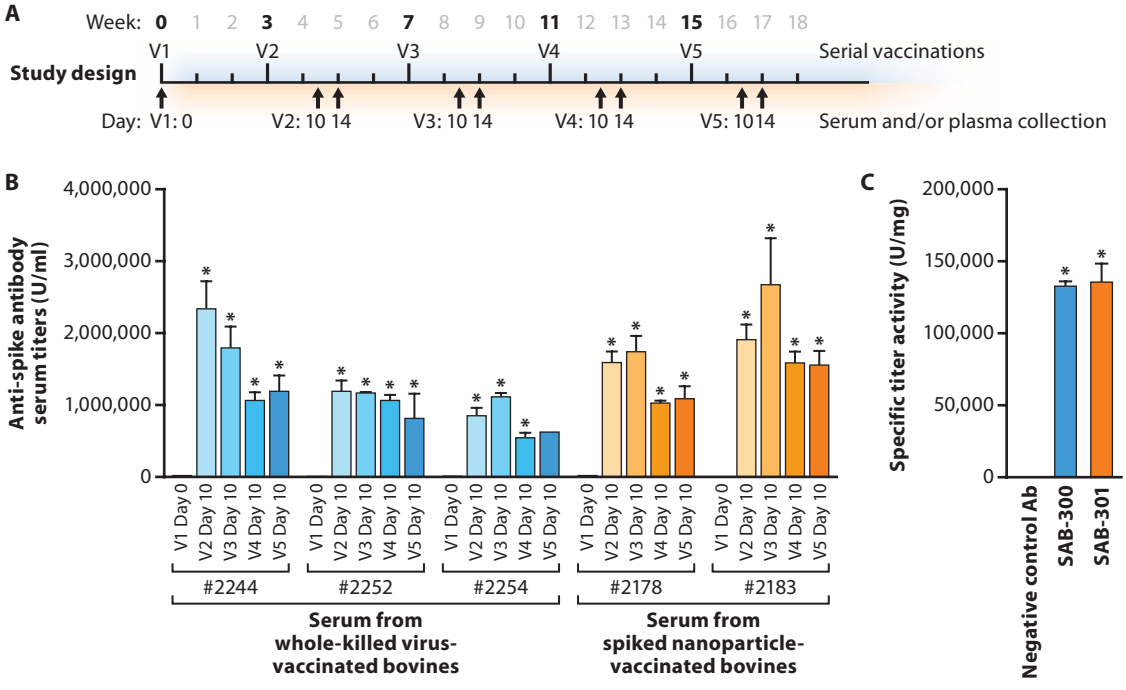

Fig. 1. MERS-CoV Tc hIgG production and ELISA titers. (**A**) Study design diagram shows events. Large ticks denote vaccine administration (V1 to V5) to Tc bovines ($n = 5$). Arrows denote collection of serum and/or plasma. Double arrows on days 10 and 14 after V2 to V5 indicate high-volume plasma collection. (**B**) Mean MERS-CoV anti-spike ELISA antibody titers (U/ml) plus SD in Tc bovine serum samples resulting in an optical density at 450 nm (OD_{450}) reading 2.5-fold higher than blank (see table S1A). *$P \leq 0.0003$, significant difference between V2 to V5 versus pre-V1D0 serum (Dunnett's test following ANOVA; see table S1B). Tc bovine #2254 had a single measurement at V5 that precluded statistical testing. (**C**) Mean MERS-CoV ELISA antibody (Ab) titers of SAB-300 and SAB-301 plus SD versus negative control Tc hIgG. *$P <$ 0.0001, significant difference between SAB-300 or SAB-301 versus negative control Tc hIgG (Dunnett's test following ANOVA; see table S1, C and D). For (B) and (C), all samples but #2254 V5 serum were independently tested in duplicate or more ($n \geq 2$).

Assays of neutralizing activity

Tc bovine sera after V2 to V5 vaccinations, SAB-300, and SAB-301 were assayed for their ability to neutralize MERS-CoV (Jordan strain) in vitro using a fluorescence-reduction neutralizing assay-50% ($FRNA_{50}$; Fig. 2, A and B, and table S2, A and B) and a tissue culture infectious dose-50% ($TCID_{50}$) assay (Fig. 2C and table S2C) in Vero E6 cells. The $FRNA_{50}$ assesses cell infection using fluorescence, and the $TCID_{50}$ assay assesses cell viability by cytopathic effect endpoint quantification. Neutralizing antibodies were found in the V2 to V5 serum of vaccinated Tc bovines. A trend was noted that a greater quantity (μg) of antibodies were required to reach $FRNA_{50}$ after V2 or V3 for both groups of bovines vaccinated with SPNV or WKVV (Fig. 2A). A greater quantity of SAB-301 antibodies were required to reach $FRNA_{50}$ compared to anti-spike control antibody ($P < 0.0001$, Dunnett's test following ANOVA; Fig. 2B and table S2B). Several potential causes for this phenomenon have been considered and are presently under investigation.

The $TCID_{50}$ assay was performed with SAB-300, SAB-301, and a Tc hIgG negative control antibody up to a dilution of 1:16,000 (Fig. 2C and table S2C). The $TCID_{50}$ of MERS-CoV infection in Vero E6 cells was below the limit of detection (158 $TCID_{50}$/ml, 0.04% of media alone control) when MERS-CoV was pretreated with SAB-300 antibodies before cell culture at all dilutions ($P = 0.0002$ for SAB-300 versus control Tc hIgG). SAB-301 was below the limit of detection at all dilutions except for the 1:8000 and 1:16,000 dilutions ($P = 0.001$ for SAB-300 versus control Tc hIgG). Nonspecific negative control Tc hIgG had no effect at any dilution. These data confirm that SAB-300 and SAB-301 contain high levels of neutralizing polyclonal antibodies. Previous studies indicate that 1 to 5% of polyclonal antibodies bind to the antigen of interest, but the percentage of SAB-300 or SAB-301 antibodies that are neutralizing has not been determined.

ADE assay

Raji cells, an immortalized human B cell line, do not express hDPP4 receptors and are not permissive to MERS-CoV infection. Therefore, Raji cells were tested to see if the presence of anti–MERS-CoV antibodies could allow for intracellular uptake, transcription of viral RNA, and the release of live virus. MERS-CoV (Jordan strain) was pretreated with SAB-300, SAB-301, or negative control Tc hIgG and then added to Raji cells and incubated for 48 hours. RNA was extracted from the cell culture and analyzed by TaqMan real-time polymerase chain reaction (RT-PCR) with primers specific for viral genomic RNA (Fig. 3A and table S3, A, B, and D). No significant differences in leader primer RNA from mock control, SAB-300, or SAB-301 were observed ($P > 0.05$, Dunnett's t test following ANOVA; Fig. 3B and table S3, A, C and D). The results indicate that Raji cells are not subject to ADE of infection in the presence of SAB-300 or SAB-301 antibodies. Preincubation of MERS-CoV Jordan strain with SAB-300 or SAB-301 antibodies did not substantially affect the production of MERS-CoV specific RNAs.

Table 1. Human IgG subclass proportions of SAB-300, and SAB-301 antibodies. Human IVIG, SAB-300, and SAB-301 were analyzed for the IgG subclass proportions by ELISA. Percentage of each subclass is presented as compared to the positive control. Chimeric antibodies (containing human κ and bovine heavy chain) were less than 1%, and other bovine proteins were less than 100 ppm.

Antibody	IgG1 (%)	IgG2 (%)	IgG3 (%)	IgG4 (%)
Human IVIG*	67	26	2.6	2.5
SAB-300	99	0.27	0.13	0.08
SAB-301	93	6.2	0.07	0.05

*Human-derived IVIG served as the positive control and was run on the same IgG subclass ELISA plates with the other antibodies.

Prophylaxis and treatment of Ad5-hDPP4–transduced mice

Laboratory mice are refractory to MERS-CoV infection (*40*). However, when transduced with an adenovirus expressing the hDPP4 receptor, mice become susceptible to MERS-CoV infection (*41*). Using this animal model, we evaluated SAB-301 for prophylactic activity. SAB-301 was selected for in vivo evaluation due to its in vitro profile and the safety and ease of producing the SNVV versus WKVV. Four groups of BALB/c mice (6 to 8 weeks old, $n = 9$ per group) were transduced intranasally with 2.5×10^8 plaque-forming units (PFU) of Ad5-hDPP4 in 75 μl of phosphate-buffered saline (PBS). At 5 days after transduction, the groups of mice were injected once intraperitoneally with 100 or 500 μg (≈5 or 25 mg/kg) of SAB-301 or negative control Tc hIgG (500 μg) or were left untreated. Twelve hours later, the mice were infected intranasally with 1×10^5 PFU of MERS-CoV–Erasmus Medical Center 2012 strain (EMC/2012) in a total volume of 50 μl. At 1, 3, and 5 days after infection, three mice per group were euthanized, and lungs were dissected. To obtain virus titers, lungs were homogenized in PBS using a manual homogenizer and clarified by centrifugation. MERS-CoV in the homogenates was detected by titration on Vero 81 cells.

We detected 5.95 to 6.27 $\log_{10}$ PFU/g of MERS-CoV at days 1 and 3 after infection in the lungs of the no-treatment group and the group that received the control Tc hIgG (Fig. 4A and table S4A). Titers were ≈10- or 200-fold lower in mice that received 100 μg ($P = 0.0001$, Dunnett's test following ANOVA) or 500 μg of SAB-301, respectively ($P < 0.0001$, Dunnett's test following ANOVA), at day 1 after infection compared to that observed in the control Tc hIgG group. Titers were also ≈10- or 200-fold lower in mice that received 100 μg ($P = 0.0014$, Dunnett's test following ANOVA) or 500 μg of SAB-301 ($P < 0.0001$, Dunnett's test following ANOVA) at day 1 after infection compared to the no-treatment group. By day 3 after infection with MERS-CoV, virus titers were reduced ≈1000-fold for the 100 μg group, whereas titers in the mice receiving 500 μg were ≈5000-fold lower compared to the Tc hIgG control and no-treatment groups ($P < 0.0001$ for all comparisons, Dunnett's test following ANOVA). At 5 days after infection with MERS-CoV, viral titers in both treatment groups (100 and 500 μg of SAB-301) were below the level of detection, whereas the Tc hIgG control and no-treatment groups had titers of 4.44 to 4.62 $\log_{10}$ PFU/g ($P < 0.0001$ for all comparisons, Dunnett's test following ANOVA). These data demonstrate that SAB-301 antibodies were able to protect mice from MERS-CoV infection with a single prophylactic injection.

We then evaluated SAB-301 for post-exposure therapeutic activity. Four groups of BALB/c mice (6 to 8 weeks, $n = 4$) were transduced intranasally with 2.5×10^8 PFU of Ad5-hDPP4 in 75 μl of PBS as before. At 5 days after transduction, mice were intranasally infected with 1×10^5 PFU of MERS-CoV in a total volume of 50 μl. Mice were then treated by intraperitoneal injection with a single 500-μg dose of SAB-301 antibodies, negative control Tc hIgG, or with no treatment at 24 hours or 48 hours after infection with MERS-CoV (Fig. 4B and table S4B).

At 5 days after MERS-CoV exposure, the mice were euthanized, and lungs were dissected. To obtain virus titers, lungs were homogenized

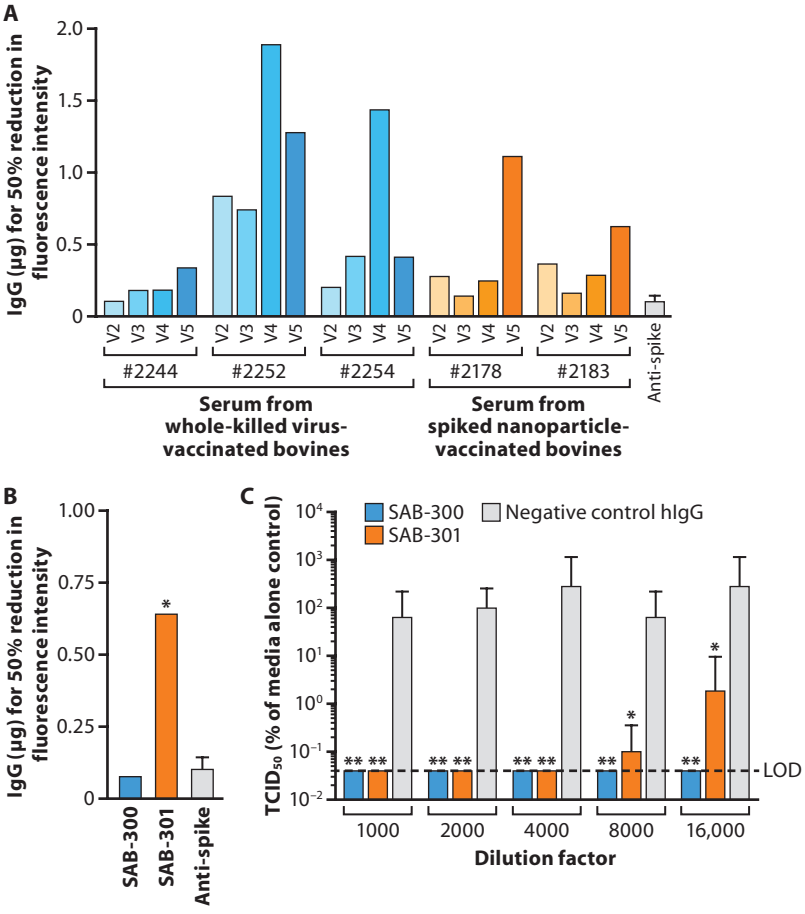

Fig. 2. MERS-CoV neutralization assays. (**A**) Sera from vaccinated (V2 to V5) bovines (n = 5) were evaluated for the quantity of neutralizing antibody (μg) that inhibited MERS-CoV infection in Vero E6 cells as measured by $FRNA_{50}$. Anti-spike rabbit Ig served as a positive control (see table S2A). (**B**) SAB-300 and SAB-301 antibodies were evaluated for the quantity of neutralizing antibody (μg) that inhibited infection of MERS-CoV in Vero cells as indicated by $FRNA_{50}$ versus positive control anti-spike rabbit Ig. *P < 0.001, significant difference between SAB-301 and anti-spike control (Dunnett's test following ANOVA; see table S2B). (**C**) Serial dilutions of SAB-300 and SAB-301 and negative control Tc hIgG were evaluated for the ability to neutralize MERS-CoV by $TCID_{50}$ assay (LOD 158 $TCID_{50}$/ml or 0.04% of media alone control) (see table S2C). Higher percentage means less inhibition of infection. *$P \leq 0.0003$, significant difference between samples and media alone; **P = 0.0002, significant difference between SAB-301 and Tc hIgG control. Below LOD and no P value is calculable (Dunnett's test following ANOVA; see table S2C). SD is given. For (A) to (C), all samples were independently tested in triplicate or more ($n \geq 3$).

in PBS using a manual homogenizer and clarified by centrifugation. MERS-CoV in the homogenates was detected by titration on Vero 81 cells. We detected 4.83 to 4.88 $\log_{10}$ PFU/g MERS-CoV titers in the lungs of the no-treatment group or the group receiving negative control Tc hIgG (Fig. 4B and table S4B). For mice injected with 500 μg of SAB-301 antibodies at 24 hours after infection, MERS-CoV lung titers were below the level of detection in comparison to that observed with untreated controls or with control Tc hIgG–treated mice (P < 0.0001 for all comparisons, Dunnett's test following ANOVA). When SAB-301 antibodies were administered 48 hours after infection, viral titers were reduced ≈1000-fold by day 5 compared to that observed with untreated control or control Tc hIgG groups (P < 0.0001 for all comparisons, Dunnett's test following ANOVA). These data demonstrate that SAB-301 antibodies were able to reduce MERS-CoV viral titers with a single therapeutic injection at either 24 or 48 hours after infection with MERS-CoV.

DISCUSSION

The use of passive immunotherapy to effectively prevent and treat infectious diseases has a long history and has been proposed as a treatment option for new and emerging pathogens (*29*). Currently, passive immunotherapy products are either polyclonal antibodies, derived from human or animal plasma donors, or monoclonal antibodies (one or cocktails of monoclonal antibodies). All of these passive immunotherapy modalities can be effective, but each modality has limitations. Human-derived IgG products (or convalescent plasma) require the identification and recruitment of willing and suitable human donors in sufficient numbers. Animal-derived IgG products have the limitation of the potential for severe reactions/toxicity after first use or after repeated administration primarily due to the human immune response to heterotypic animal antibodies. Monoclonal antibodies can require lengthy development processes, be costly to scale up, and result in the rapid development of escape mutants (*42*–*44*), as seen with SARS-CoV (*45*, *46*), compared to that observed with polyclonal antibodies. Alternative methods to produce passive immunotherapy without the limitations of animal/human IgGs and monoclonal antibody therapeutics would be beneficial.

Tc bovines offer a possible production platform to rapidly produce fully human, polyclonal, and polypathogen IgG in large quantities for the prevention and treatment of human diseases (*35*, *36*). Because polyclonal antibodies target multiple epitopes, viral escape leading to a pathogenic virus is more difficult. Viruses that escape from a single antibody may still maintain high fitness, whereas escape from multiple antibodies has been shown to lead to reduced fitness in vitro and in vivo. Experimental high-titer Tc hIgGs have shown in vivo protection and/or in vitro neutralization against bivalent hantavirus (Andes and Sin Nombre viruses) (*35*), bivalent Ebolavirus (Zaire and Sudan viruses) (*34*), and trivalent seasonal influenza A viruses (pH1N1, H3N2, type B), trivalent alphaviruses (Venezuelan, eastern, and western equine encephalitis viruses), and tetravalent dengue viruses 1 to 4 are also under evaluation. We have shown that the WKVV and SPNV vaccines are highly immunogenic in Tc bovines, and the resulting anti–MERS-CoV Tc hIgG are potent in vitro and in transduced mice. We attempted to infect and treat marmosets but failed to cause patent MERS-CoV infection as previously reported (*47*, *48*).

Our rationale for selecting SAB-301 over SAB-300 antibodies for the in vivo treatment studies and hence further preclinical development is due to the ease, cost, safety, and production capability of SPNV versus WKVV (for example, biosafety level-3 laboratory and

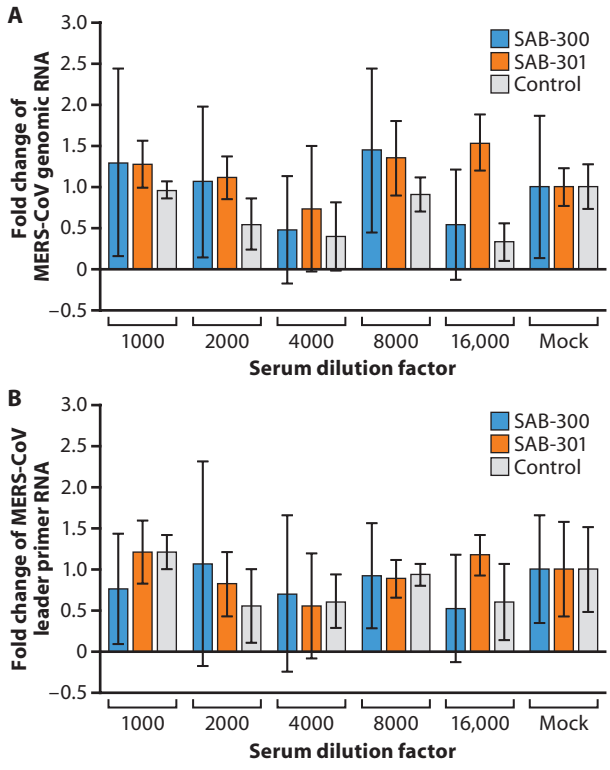

Fig. 3. ADE assay. RT-PCR analysis of MERS-CoV mRNA from nonpermissive Raji cells that were preincubated with serum containing SAB-300, SAB-301, negative control Tc hIgG or with mock control and then infected with MERS-CoV. At 48 hours after infection, RNA was isolated and assayed for amount of MERS-CoV–specific transcript by TaqMan RT-PCR. (**A**) Using primers specific for the E (UpE) gene, fold change and SD of MERS-CoV–specific genomic RNA are compared to those observed with mock samples (see table S3, A, B, and D). (**B**) MERS-CoV replicating RNA is analyzed with primers specific for the leader primer region (see table S3, A, C, and D). SD is given, and all samples were independently tested in triplicate ($n = 3$). No significant differences from mock control were observed ($P > 0.05$, all comparisons, Dunnett's test following ANOVA).

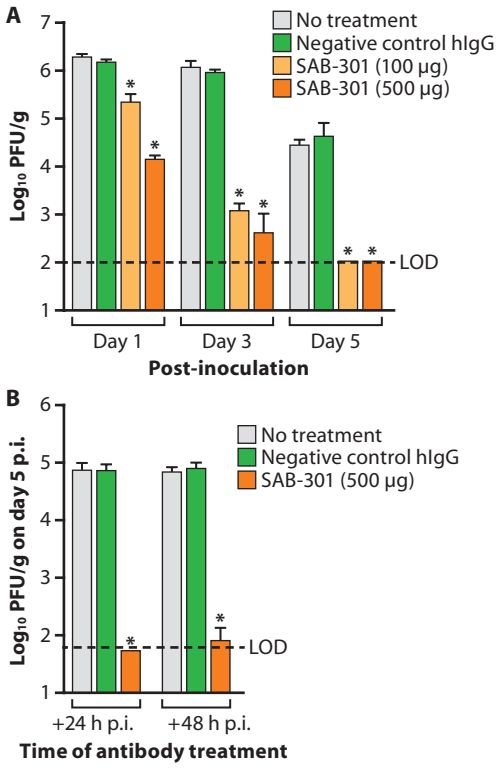

Fig. 4. Inhibition of MERS-CoV replication in vivo. SAB-301 antibodies were injected into Ad5-hDPP4–transduced BALB/c mice ($n = 3$ per group per time point) intraperitoneally. (**A** and **B**) Mice received SAB-301 (A) 12 hours before or (B) 24 or 48 hours after intranasal infection with 1×10^5 PFU of MERS-CoV–EMC/2012 strain. Virus titers ($\log_{10}$ PFU/g tissue) in the lungs and SD were measured at days 1, 3, and 5 after infection in (A) and at day 5 after infection in (B). Mice receiving 100 or 500 µg of SAB-301 12 hours before challenge had significantly lower lung titers ($P < 0.0001$) on days 1, 3, and 5 after infection compared to that observed with untreated controls or to control Tc hIgG (Dunnett's test following ANOVA). Mice receiving SAB-301 antibodies 24 or 48 hours after MERS-CoV challenge had significantly lower lung viral titers at day 5 after infection compared to untreated controls or to control Tc hIgG ($P \leq 0.0001$, Dunnett's test following ANOVA). All samples were independently tested in duplicate or more ($n \geq 2$).

manufacturing facilities). SAB-301 antibodies are primarily human IgG1 and are produced with a series of steps designed to eliminate adventitious agents and bovine proteins. This purification process should reduce the potential for adverse reactions seen with other animal-derived IgG products. Additionally, IgG1 is a potent activator of antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and the high concentrations of IgG1 in SAB-301 antibodies could potentially enhance neutralization of MERS-CoV in a human recipient in vivo (*49*). Therefore, we propose that SAB-301 could be further assessed in preclinical and possibly clinical studies to treat MERS-CoV infections.

However, this study has limitations. No Tc hIgG has been evaluated in humans, and results from the in vitro assays or from use of Tc hIgG in an in vivo Ad5-hDPP4 murine model may not accurately predict safety or efficacy in a human host. In particular, the interaction of polyclonal Tc bovine human IgG1 antibodies with the Fc receptors on mouse effector cells (ADCC) and with mouse complement (CDC) has not been studied. What was observed in mice could be the result of direct neutralization of the virus without substantial ADCC/CDC activity. Humans should have substantially more ADCC/CDC activity than mice, but the safety and efficacy of this enhanced activity cannot be determined in animal models. Additionally, although we relatively rapidly developed and tested new anti–MERS-CoV therapeutics, advancing SAB-301 antibodies into clinical trials will require funding and support.

MATERIALS AND METHODS

Study design

The overall objective of the study was to determine whether Tc hIgG purified from the plasma of animals vaccinated against MERS-CoV could neutralize virus in vitro and in vivo. The bovines were not randomized but were carefully selected for health and genetic characteristics. Experimental approaches used for each experiment were

previously used with other viruses (*32*, *34*, *35*, *41*). Purified, diluted Tc hIgGs were added to Vero E6 cells, exposed to MERS-CoV Jordan strain, and assayed for ability to neutralize MERS-CoV. BALB/c mice were randomized to four treatment groups: one negative control group (Tc hIgG), no intervention, and treatment with 100 or 500 μg of SAB-301 12 hours before intranasal exposure to EMC/2012 strain of MERS-CoV. In a second experiment, mice were randomized to four treatment groups: one negative control group (Tc hIgG), no intervention, and treatment with SAB-301 24 or 48 hours after intranasal exposure to MERS-CoV (EMC/2012 strain). Sample sizes for the mouse experiments were determined by indications of a likely response obtained from a previous study (*41*). Blinding of researchers and technicians was not done. Replication numbers for each experiment are provided in the figure legends and/or the Supplementary Materials.

Tc bovines

Tc bovines were produced as previously described (*32*, *36*–*38*). Briefly, the Tc bovines used in this study are homozygous for triple knockouts in the endogenous bovine Ig genes (*IGHM*$^{-/-}$ *IGHML1*$^{-/-}$ *IGL*$^{-/-}$) and carry a HAC vector with an IgG1 production bias, labeled as KcHACD. See Supplementary Materials and Methods for details.

Inactivated whole virion vaccine

MERS-CoV (4×10^9 PFU of Jordan-N3/2012 strain) was grown on Vero CCL-81 cells, isolated, and irradiated with a cobalt source until a dose of 60 kGy was achieved. The material was tested for inactivation before release from a biosafety level-3 laboratory for vaccination. See Supplementary Materials and Methods for details.

Spike protein nanoparticle vaccine

The antigen is a recombinant glycoprotein nanoparticle to the Al-Hasa 1 2013 MERS-CoV strain (National Center for Biotechnology Information accession #AGN70962) that is codon-optimized for optimal expression in insect cells lines and purified by a previously described method (*50*).

Vaccination of Tc bovines

Three Tc bovines in group 1 (#2244, #2252, and #2254) were immunized with WKVV at a 1×10^8 to 2×10^8 PFU/dose formulated with SAB's proprietary adjuvant formulation (SAB-adj-2). Two Tc bovines in group 2 (#2178 and #2183) were immunized with SPNV (2 mg/kg) formulated with SAB's proprietary adjuvant formulation (SAB-adj-1) The Tc bovines in both groups were vaccinated five times (V1 to V5) at 3- to 4-week intervals.

Plasma collection and Tc hIgG production

Before V1, plasma was collected from each Tc bovine to serve as the negative control. Up to 2.1% of body weight of hyperimmune plasma per animal was collected from immunized Tc bovines on days 10 and 14 after vaccinations V2 to V5. Plasma was collected using an automated plasmapheresis system (Autopheresis-C, model A-200, Baxter Healthcare), pH-adjusted, fractionated by caprylic acid, and centrifuged. The supernatant was purified by anti-human IgG light chain–specific column, KappaSelect (GE Healthcare Life Sciences). See Supplementary Materials and Methods for details.

MERS-CoV spike protein–specific ELISA

Determination of MERS-CoV spike protein–specific human IgG antibody titers was performed in MaxiSorp Immuno 96-well ELISA plates (Thermo Scientific) coated overnight at 4°C with recombinant MERS-CoV spike protein (2 μg/ml) (Al-Hasa strain) in PBS at 100 μl per well. After blocking, serial dilutions of serum samples or SAB-300 or SAB-301 antibodies were added to the plate. Diluted goat anti-human IgG-Fc conjugated with horseradish peroxidase (Bethyl) was added to the plates, and bound anti-spike antibodies were detected colorimetrically by using the 3,3′,5,5′-tetramethylbenzidine substrate kit (Kirkegaard & Perry Laboratories). See Supplementary Materials and Methods for details.

Human IgG subclass proportion by ELISA

For detection of human IgG1, IgG2, IgG3, or IgG4 composition of SAB-300 and SAB-301 antibodies, human IgG subclass ELISAs were performed in MaxiSorp Immuno 96-well ELISA plates (Sigma-Aldrich) in accordance with the anti-human IgG subclass antibody manufacturer's instructions. See Supplementary Materials and Methods for more details.

MERS-CoV exposure studies in transduced adenovirus/hDPP4 mice

Transduced adenovirus/hDPP4 BALB/c mice were infected with EMC/2012 strain of MERS-CoV as described (*41*). BALB/c mice ($n = 3$ per group) were injected intraperitoneally with 100 or 500 μg (5 to 25 mg/kg) of negative control or test Tc hIgG (SAB-301). Mice were infected intranasally with MERS-CoV (1×10^5 PFU) in a total volume of 50 μl 12 hours before or 24 and 48 hours after administration of the Tc hIgG. To obtain MERS-CoV titers, lungs were homogenized, and virus was titrated on Vero 81 cells. See Supplementary Materials and Methods for more details.

Source of mice

Specific pathogen–free 6- to 8-week-old BALB/c laboratory mice were purchased from the National Cancer Institute and maintained in the animal care facility at the University of Iowa. All murine protocols were approved by the University of Iowa International Animal Care and Use Committee.

$FRNA_{50}$ assay

$FRNA_{50}$ assays were performed to determine neutralizing activity of anti–MERS-CoV antibody samples. Vero E6 cells (8×10^4 cells per well) were plated on 96-well Operetta-compatible plates (PerkinElmer). Serum samples were heat-inactivated, diluted serially 1:40 to 1:327,680 in Dulbecco's modified Eagle's medium (Lonza). MERS-CoV virus stock was diluted to 0.96 PFU/μl using serum-free Dulbecco's modified Eagle's medium (Lonza). Virus + serum mixture was added to the 96-well plates and incubated for 24 hours at 37°C and 5% CO_2. Negative control (no virus and no serum) samples and positive control [virus + antigen affinity-purified rabbit polyclonal antibody to MERS-CoV spike protein #40069-RP02 (anti-spike), Sino Biological] samples were used. Cells were fixed by adding 20% neutral-buffered formalin and were processed by labeling with a primary MERS-CoV anti-spike antibody. A secondary antibody goat anti-rabbit IgG (heavy and light chain) conjugated to Alexa Fluor 594 (A-11037, Thermo Fisher Scientific) detected the primary antibody and was visualized on Operetta High Content Imager (PerkinElmer). The dilution at which 50% inhibition of relative fluorescence intensity was observed was reported as the $FRNA_{50}$. See Supplementary Materials and Methods for more details.

$TCID_{50}$ assay

The $TCID_{50}$ assay was performed as previously described (*50*). See Supplementary Materials and Methods for more details.

ADE assay

To test for ADE of MERS-CoV infection, 1×10^4 Raji or Vero E6 cells per well were seeded into 96-well plates (Corning) and cultured overnight. MERS-CoV–Jordan strain at a $TCID_{50}$ of 10,000 was added to SAB-300 or SAB-301 antibodies, incubated at 37°C for 1 hour, and added to Raji or Vero E6 cells. At 48 hours after infection, supernatant was collected and frozen at −80°C until infectivity was assessed by $TCID_{50}$ assay as previously described (*51*).

RNA was extracted from Raji cell pellets using the PureLink RNA Mini Kit (Life Technologies) according to the manufacturers' instructions. Genome copies of MERS-CoV RNA were measured using the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems) according to the manufacturers' instructions using a triplex of primers obtained from Life Technologies. PCRs in MicroAmp Fast Optical Reaction Plates (Applied Biosystems) were read on 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems), and data were analyzed using the ΔC_t (cycle threshold) method (*52*). See Supplementary Materials and Methods for more details.

Cells and viruses

See Supplementary Materials and Methods.

Statistical analyses

We used one-way ANOVA followed by Dunnett's multiple comparisons test versus control using GraphPad Prism 6.0 for comparison of groups throughout the paper. The testing level was $\alpha = 0.05$ and two-sided. GraphPad Prism 6.0 does not provide an exact *P* value when less than 0.0001. When the raw data violated the equal variance assumption of ANOVA as indicated by the Brown-Forsythe test, data were log-transformed for analysis. Namely, the test compared antibody titers after V2 to V5 to pre-V1, specific titer activity of SAB-300 or SAB-301 to negative control antibody, mass of IgG for $FRNA_{50}$ of sera samples from V2 to V5 to the positive control anti-spike antibody, mass of IgG for $FRNA_{50}$ of SAB-300 and SAB-301 to positive control anti-spike antibody, $TCID_{50}$ of SAB-300 or SAB-301 to negative control (Tc hIgG), lung titers of mice receiving SAB-301 to negative control Tc hIgG and no treatment groups, and lung titers of mice receiving SAB-301 after exposure to untreated controls at day 5 after infection. The statistical approaches are valid according to C. Olsen (Department of Preventive Medicine and Biostatistics, Uniformed Services University).

SUPPLEMENTARY MATERIALS

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Materials and Methods
Tables S1A. Source data for Fig. 1B.
Table S1B. *P* value of Dunnett's test (V2, V3, V4, or V5 versus pre-V1D0) for Fig. 1B.
Table S1C. Specific titer activity of purified antibodies for Fig. 1C.
Table S1D. *P* value of Dunnett's test (SAB-300 or SAB-301 versus negative control) for Fig. 1C.
Table S2A. Source data for Fig. 2 (A and B).
Table S2B. *P* value of Dunnett's test for anti-spike antibody versus SAB-300 and SAB-301 for Fig. 2B.
Table S2C. Source data and *P* value of Dunnett's test for Fig. 2C.
Table S3A. Source data for Fig. 3 (A and B).
Table S3B. Source data for Fig. 3A.
Table S3C. Source data for Fig. 3B.
Table S3D. *P* value of Dunnett's test for Fig. 3 (A and B).
Table S4A. Source data for Fig. 4A.
Table S4B. Source data for Fig. 4B.

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Human polyclonal immunoglobulin G from transchromosomic bovines inhibits MERS-CoV in vivo

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Emerging therapeutics

The ability to treat emerging infections, such as the Middle East respiratory syndrome coronavirus (MERS-CoV), has been limited by the turnaround time of developing new therapeutics. Now, Luke *et al*. report that transchromosomal bovines can rapidly produce large quantities of fully human polyclonal IgG antibodies to MERS-CoV after vaccination. These antibodies could neutralize MERS-CoV both in vitro and clear infection in mice in vivo. Human testing will confirm whether passive immunization with these antibodies can safely and effectively treat infection in infected individuals.

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