

# MERS-CoV–Specific T-Cell Responses in Camels after Single MVA-MERS-S Vaccination

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We developed an ELISPOT assay for evaluating Middle East respiratory syndrome coronavirus (MERS-CoV)–specific T-cell responses in dromedary camels. After single modified vaccinia virus Ankara-MERS-S vaccination, seropositive camels showed increased levels of MERS-CoV–specific T cells and antibodies, indicating suitability of camel vaccinations in disease-endemic areas as a promising approach to control infection.

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a betacoronavirus that is of special interest for public health. Dromedary camels have been identified as natural animal reservoirs, with >90% MERS-CoV seroprevalence reported in Middle East countries (1–4). Such permanent viral circulation within camel herds poses a constant threat of zoonotic transmission into human populations (5). Thus, a potentially useful approach to prevent MERS-CoV zoonoses focuses on vaccination-based reduction of spill over events from camels as a classical One Health approach (6,7).

Besides antibody responses, MERS-CoV–specific T cells probably play a major role in rapid viral clearance and long-lasting immunity against MERS-CoV infections (8). Although serologic assays were rapidly developed, established T-cell assays for camels are still lacking, yet urgently needed for contact tracing, epidemiology, and vaccine evaluation studies. Several MERS-CoV–specific vaccine candidates are under investigation and use different platforms,

such as DNA vaccines or adenoviral vectors (9–12). A promising experimental vaccine for use in camels is recombinant modified vaccinia virus Ankara (MVA) expressing full-length MERS-CoV spike protein as antigen (MVA-MERS-S) (13). Experimental vaccination with MVA-MERS-S in dromedaries can induce protective immunity to MERS-CoV (14). Moreover, MVA-MERS-S proved safe and immunogenic in a phase Ia/b clinical study in humans (15). The aim of this exploratory study in Dubai, United Arab Emirates, where enzootic MERS-CoV is prevalent, was to establish an assay for detecting MERS-CoV–specific T cells in dromedary camels under field conditions.

## The Study

To investigate the effect of MVA-MERS-S vaccination in naive or previously infected animals, we divided 12 adult dromedary camels into 2 cohorts: naive and MERS-CoV seropositive solely based on presence of MERS-S IgG (by ELISA) before vaccination. Eight camels had antibody titers relevant for seroconversion (optical density [OD] ratio >1.1), indicating previous MERS-CoV infection, whereas the remaining 4 camels had no MERS-specific antibodies (Table 1).

**Table 1.** MERS-CoV seroprevalence in 12 dromedary camels before vaccination, Dubai, United Arab Emirates\*

Camel ID	Optical density ratio (ELISA)	MERS-CoV infection status/cohort
1	0.07	Naive
2	2.09	Seropositive
3	2.97	Seropositive
4	4.00	Seropositive
5	0.06	Naive
6	0.07	Naive
7	0.05	Naive
8	4.11	Seropositive
9	3.42	Seropositive
10	4.59	Seropositive
11	2.48	Seropositive
12	3.22	Seropositive

\*ID, identification; MERS-CoV, Middle East respiratory syndrome coronavirus.

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**Table 2.** Cohorts of dromedary camels by MERS-CoV seroprevalence and vaccine candidate used Dubai, United Arab Emirates\*

Category	Type	Vaccine candidate		Total
		MVA	MVA-MERS-S	
MERS-CoV infection	Naive	1	3	4
	Seropositive	3	5	8
	Total	4	8	12

\*MERS-CoV, Middle East respiratory syndrome coronavirus; MVA, modified vaccinia virus Ankara; MVA-MERS-S, modified vaccinia virus Ankara expressing full-length MERS-CoV spike protein as antigen.

Camels were either vaccinated with MVA-MERS-S or MVA as a control by using intramuscular inoculation (dose  $2.5 \times 10^8$  PFU/2 mL) (Table 2). Animals and application sites were monitored and scored daily for an observation period of 22 days. No clinical signs or potential side effects were observed (data not shown). Analysis of the IgG response at the day of vaccination and 15 days later (Figure 1) showed no differences in MERS-CoV–specific antibodies in naive camels (MVA– and MVA-MERS-S–vaccinated camels).

One seropositive animal vaccinated with MVA showed an increased optical density (OD) ratio of 0.54, whereas the other 2 animals showed no difference or a decreased ratio of 0.19. Seropositive camels vaccinated with MVA-MERS-S ( $n = 5$ ) mounted increased levels of MERS binding antibodies, with a mean titer (OD ratio) of 4.44 on day 15 compared with 3.22 at day 0 postvaccination. Two MVA-MERS-S vaccinated camels from seropositive animals showed an increased OD ratio  $>2.4$ .

To assess T-cell responses, we prepared peripheral blood mononuclear cells (PBMCs) from blood plus EDTA on different days postvaccination during the observation period. PBMCs were restimulated with 2 pools of overlapping peptides comprising either the S1 or S2 subunit of MERS-CoV spike glycoprotein (Appendix Figure, <https://wwwnc.cdc.gov/EID/article/29/6/23-0128-App1.pdf>) analyzed by using interferon (IFN)  $\gamma$  ELISpot assays.

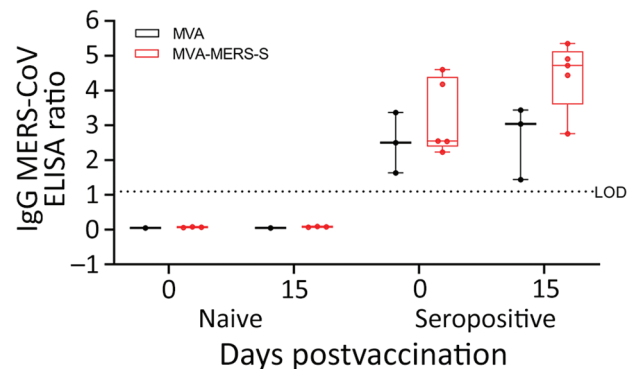
After S1 peptide pool stimulation, we detected no IFN- $\gamma$ –producing cells in the MVA-vaccinated naive animals (Figure 2, panel A). MVA-MERS-S vaccinated naive animals ( $n = 3$ ) showed detectable levels of S1-specific T cells on day 6 postvaccination (mean 11.1 spot-forming T cells [SFC]/ $10^6$  PBMCs), which further increased until day 8 postvaccination (mean 63.3 IFN- $\gamma$  SFC/ $10^6$  PBMCs).

MVA-vaccinated seropositive animals showed negligible levels of IFN- $\gamma$ –producing cells, except for 1 animal that had S1-specific T cells on days 6 and 8 postvaccination (mean 217.8 IFN- $\gamma$  SFC/ $10^6$  PBMCs). Seropositive MVA-MERS-S–vaccinated animals had substantially higher activated S1-specific T-cell levels starting on day 6 postvaccination (mean 230.6 IFN- $\gamma$  SFC/ $10^6$  PBMCs), further increasing on day 8 postvaccination (mean 497.8 IFN- $\gamma$  SFC/ $10^6$  PBMCs).

Subsequently, S1-specific T-cell levels decreased on day 10 (mean 110 IFN- $\gamma$  SFC/ $10^6$  PBMCs), until reaching relatively low levels at day 22 postvaccination (mean 42.8 IFN- $\gamma$  SFC/ $10^6$  PBMCs).

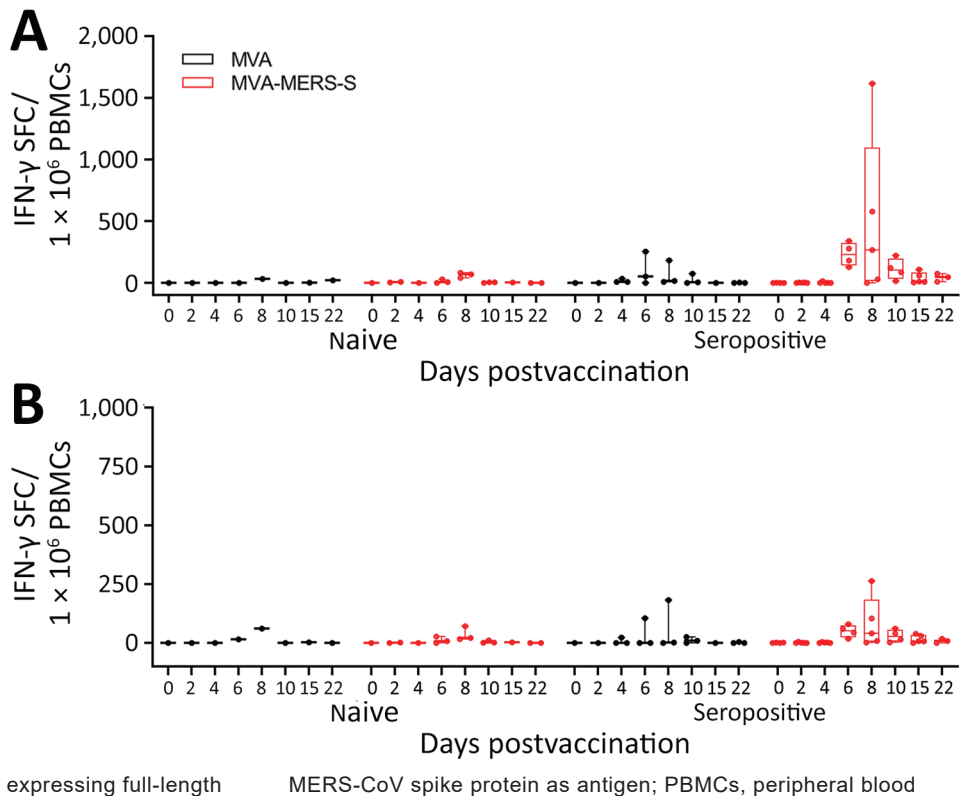
Upon S2 peptide stimulation, we detected lower levels of IFN- $\gamma$ –producing cells compared with S1 peptide stimulation (Figure 2, panel B). The MVA-vaccinated naive camels had low levels of IFN- $\gamma$ –producing cells on day 6 and 8 postvaccination (mean 38.3 IFN- $\gamma$  SFC/ $10^6$  PBMCs). MVA-MERS-S–vaccinated naive animals showed low responses in all animals; mean levels of 11.9 IFN- $\gamma$  SFC/ $10^6$  PBMCs on day 6 postvaccination increased to 36.3 IFN- $\gamma$  SFC/ $10^6$  PBMCs on day 8 postvaccination, then decreased again by day 22 postvaccination.

Two MVA-vaccinated seropositive animals mounted no detectable levels of S2-specific T cells. The same seropositive animal mounting S1-specific T cells revealed increased levels of S2-specific T cell activation on day 6 and 8 postvaccination (mean



**Figure 1.** Antigen-specific humoral immunity after MVA-MERS-S vaccination in dromedary camels, Dubai, United Arab Emirates. MERS-CoV seropositive and naive dromedary camels were immunized once with  $2.5 \times 10^8$  plaque-forming units of MVA-MERS-S or MVA as a vector control. Serum samples were collected on day 0 and on day 15 after single-shot vaccination. Black indicates serum samples analyzed for MERS-CoV S1 IgG by ELISA of MVA vaccinated animals and red indicates for MVA-MERS-S–vaccinated animals. Box plots show individual values (dots), median values (horizontal lines within boxes), first and third quartiles (box tops and bottoms), and minimums and maximums of value distribution (error bars). LOD, limit of detection; MERS-CoV, Middle East respiratory syndrome coronavirus; MVA, modified vaccinia virus Ankara; MVA-MERS-S, modified vaccinia virus Ankara expressing full-length MERS-CoV spike protein as antigen.

**Figure 2.** Antigen-specific cellular immunity after MVA-MERS-S vaccination in dromedary camels, Dubai, United Arab Emirates. PBMCs were isolated from blood samples on different days post-single-shot vaccination and IFN- $\gamma$  SFCs were measured by ELISpot assay after restimulation of PBMCs from different time points with overlapping peptides comprising the MERS-CoV S1 (A) and MERS-CoV-S2 (B) protein subunit. Box plots show individual values (dots), median values (horizontal lines within boxes), first and third quartiles (box tops and bottoms), and minimums and maximums of value distribution (error bars). IFN- $\gamma$ , interferon- $\gamma$ ; MERS-CoV, Middle East respiratory syndrome coronavirus; MVA, modified vaccinia virus Ankara; MVA-MERS-S, modified vaccinia virus Ankara expressing full-length mononuclear cells; SFCs, spot-forming T cells.



143.6 IFN- $\gamma$  SFC/ $10^6$  PBMCs). All seropositive MVA-MERS-S-vaccinated animals had levels of S2-specific T cells that increased on day 6 postvaccination (mean 50.6 IFN- $\gamma$  SFC/ $10^6$  PBMCs), further increasing on day 8 postvaccination (mean 84 IFN- $\gamma$  SFC/ $10^6$  PBMCs). Again, the S2-specific T cells subsequently decreased by day 22 postvaccination (mean 8.9 IFN- $\gamma$  SFC/ $10^6$  PBMCs).

## Conclusions

This exploratory study confirms the presence of MERS-S-specific T cells in dromedary camels after a single MVA-MERS-S vaccination under field conditions as analyzed by IFN- $\gamma$  ELISPOT assay. Previous infection seems not to hamper the practicability or value of vaccination trials because specific T cells were immunologically boosted in seropositive camels. These data are consistent with a recent study of humoral boost effects in seropositive camels after vaccination with a chimpanzee adenoviral vector-based MERS-CoV vaccine (12). This finding is relevant because serum antibodies are considered to reduce viral replication (6). MVA-MERS-S vaccination also reactivated humoral immune responses in seropositive camels. Our previous study confirmed that MERS-CoV-S-specific antibodies correlate with

reduced viral excretion in camels (14). These preliminary results could have major implications for implementing future MVA-MERS-S camel vaccination studies in disease-endemic areas.

Naive MVA-MERS-S-vaccinated animals mounted fewer MERS-CoV-S-specific T cells than seropositive animals and failed to show S-specific antibodies after single MVA-MERS-S vaccination. Thus, further optimizing MVA-MERS-S-induced immunogenicity would require modifying vaccination strategies under field conditions, such as prime-boost vaccination regimens or alternative applications including intranasal immunization.

Although it is unlikely for the specific T cells detected in 1 seropositive and 1 naive camel after MVA vaccination, we cannot rule out a field infection between vaccination and sample preparation. Rather, we hypothesize that the seropositive animal could have remounted a cellular immune response caused by MVA-induced immune activation and potential coactivation of S-peptide specific T cells from previous MERS-CoV infection. In the naive camel, which did not seroconvert or mount S1-specific responses, nonspecific reactions could explain the detection of IFN- $\gamma$  SFC.

The first limitation for this proof-of-concept study is that it was conducted as an exploratory study to

evaluate MERS-CoV–specific T cells in a few camels and provide a basis for further evaluation of camel vaccination in disease-endemic areas. To verify the potential protective capacity of vaccine-induced immune responses under field conditions, it will be essential to also characterize the infection status and demonstrate reduced virus excretion in vaccinated, subsequently infected animals. Future field studies could be based on MVA-MERS-S vaccination, not only in prime-only immunization cohorts but also in prime-boost applications, especially in juvenile animals, the probable main drivers of MERS-CoV transmission in camel populations (6). Our findings should contribute to establishing an advanced method for evaluating MERS-CoV–specific cellular immunity in dromedary camels.

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### About the Author

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# MERS-CoV–Specific T-Cell Responses in Camels after Single MVA-MERS-S Vaccination

## Appendix

### Materials and Methods

#### Vaccine Candidate

The vaccine candidate used in this proof-of-concept study is MVA-MERS-S, a recombinant Modified Vaccinia Virus Ankara, as a safe vaccine platform expressing the full-length MERS-CoV spike (S) protein. As described by Song et al. (1), the vaccine candidate was constructed as follows: cDNA with the whole gene sequence of MERS-CoV-S was provided by performing DNA synthesis (Invitrogen Life Technology, Regensburg, Germany) and modified by inserting silent mutations for vaccinia virus transcription. The MERS-CoV-S coding sequences were integrated into a MVA vector plasmid under the transcriptional control of the vaccinia virus early/late promoter PmH5. For selection of the correctly constructed recombinant MVA-MERS-S, the fluorescent marker gene mCherry was also placed in the MVA vector plasmid (under transcriptional control of the vaccinia virus late promoter P11). The MVA vector plasmid was further inserted into the deletion site Del III in the MVA genome by homologous recombination. The fluorescent marker protein mCherry introduced while cloning enabled isolation of recombinant MVA-MERS-S by using fluorescence. The mCherry marker was removed by marker gene deletion using repetitive sequences. To further characterize the vaccine candidate, PCR analysis and multiple-step growth analysis were performed, confirming the genetic integrity, stability and safety of MVA-MERS-S.

## **MVA-Based Vaccination in Dromedary Camels in Dubai**

Adult dromedary camels (n = 12) from the Central Veterinary Research Laboratory in Dubai, UAE were housed in different enclosures and had unlimited access to water and food. All of the animals underwent a comprehensive general examination and showed healthy general clinical conditions. The animals were immunized with an intramuscular single-shot-vaccination of  $2.5 \times 10^8$  PFU/2 mL. Eight of the twelve animals were vaccinated with the recombinant MVA-MERS-S and the other four animals with the non-recombinant MVA as a viral vector control.

## **IgG ELISA**

Serum samples from the selected dromedary camels were collected on the day of vaccination and 15 days post vaccination. MERS-CoV seroprevalence was analyzed by using the Anti-MERS-CoV-ELISA Camel (IgG) kit from EUROIMMUN. The commercial ELISA kit is based on the MERS-CoV S1 subunit as described by Drosten et al. (2). Diluted serum samples (1:101 in sample buffer) were first incubated at 37°C for 30 minutes in microplate wells coated with recombinant structural MERS-CoV S1 protein. For detection, an enzyme-labeled anti-camel IgG was then added to the wells in a second incubation step (37°C, 30 minutes). Color-coded substrate was finally added to the wells for 15 minutes at room temperature leading to a color reaction. In between, wash steps were included. The extinction value was measured at OD 450 nm light and semiquantitative evaluations were performed by using the ratio values (sample value extinction over calibrator value extinction). As recommended by EUROIMMUN, a ratio <0.8 was set as a negative result and a ratio  $\geq 1.1$  as positive.

## **Enzyme-Linked Immunospot (ELISpot)**

Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA blood directly after sampling by density gradient centrifugation on day 0, 2, 4, 6, 8, 10, 15 and 22 post vaccination and stored frozen. Later on, samples from different timepoints from individual camels were quickly thawed at 37°C, washed, and resuspended in RPMI 1640 medium (SIGMA-ALDRICH, Taufkirchen, Germany) supplemented with 10% heat inactivated FCS, 1% Penicillin-Streptomycin, 1% non-essential amino acids and 1% vitamins. The cells were counted with the TC20 Automated Cell Counter (Bio-Rad Laboratories, Feldkirchen, Germany) and  $3 \times 10^5$  live PBMCs per well were seeded into 96-well round bottom plates (Sarstedt, Nümbrecht, Germany) before adding the stimulants. For PBMC stimulation, peptides that can directly bind to MHC I and MHC II molecules on the APC surface were used as an established method for stimulating

both CD8 and CD4 T cells. We used two different peptide pools from the S1 and S2 domains comprising 168 overlapping peptides each (1 µg peptide/ml RPMI 1640) providing the whole spike glycoprotein of MERS-CoV (JPT Peptide Technologies, Berlin, Germany). Each peptide consists of 15 aa (15-mers) overlapping in 11 aa with the following peptide (Supplemental Figure 1). PBMCs stimulated with phorbol myristate acetate and ionomycin (SIGMA-ALDRICH, Taufkirchen, Germany) and non-stimulated cells were used as positive and negative controls. The cells were then transferred onto PVDF membrane plates previously coated with mouse anti-bovine IFN- $\gamma$  monoclonal antibody (bIFN $\gamma$ -I; Mabtech, Nacka, Sweden) and incubated for 24 hours at 37°C. After removing the inoculate, biotinylated mouse anti-bovine IFN- $\gamma$  monoclonal antibody (PAN), streptavidin-ALP and BCIP/NBT-plus substrate were added onto the ELISpot plates in this order with washing steps in between. For scanning and counting the spots we used the automated ELISpot Reader ImmunoSpot S6 ULTIMATE UV Image Analyzer (Immunospot, Bonn, Germany) and ImmunoSpot 7.0.20.1 as the reader software version.

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<u>MIHSVFLLMF</u>	<u>LLTPTESYVD</u>	<u>VGPDsvksac</u>	<u>IEVDIQQTFF</u>	<u>DKTWPRPIDV</u>	<u>SKADGHIYPQ</u>
<u>GRTYSNITIT</u>	<u>YQGLFPYQGD</u>	<u>HGDMYVVSAG</u>	<u>HATGTTQKL</u>	<u>FVANYSQDVK</u>	<u>QFANGFVVRI</u>
<u>GAAANSTGTV</u>	<u>IISPSTSATI</u>	<u>RKIYPAFLMG</u>	<u>SSVGNFSDGK</u>	<u>MGRFFNHTLV</u>	<u>LLPDGCGTLL</u>
<u>RAFYCILEPR</u>	<u>SGNHCPAGNS</u>	<u>YTSFATYHTP</u>	<u>ATDCSDGNYN</u>	<u>RNASLNSFKE</u>	<u>YFNLRNCTFM</u>
<u>YTYNITEDEI</u>	<u>LEWFGITQTA</u>	<u>QGVHLFSSRY</u>	<u>VDLYGGNMFQ</u>	<u>FATLPVYDTI</u>	<u>KYYSIIPHSI</u>
<u>RSIQSDRKAW</u>	<u>AAFYVYKLQP</u>	<u>LTFLLDFSVD</u>	<u>GYIRRAIDCG</u>	<u>FNDLSQLHCS</u>	<u>YESFDVESGV</u>
<u>YSVSSFEAKP</u>	<u>SGSVVEQAEG</u>	<u>VECDFSPLLS</u>	<u>GTPPQVYNFK</u>	<u>RLVFTNCNYN</u>	<u>LTKLLSLFSV</u>
<u>NDFTCSQISP</u>	<u>AAIASNCYSS</u>	<u>LILDYFSYPL</u>	<u>SMKSDLSVSS</u>	<u>AGPISQFNYK</u>	<u>QSFNSPTCLI</u>
<u>LATVPHNLTT</u>	<u>ITKPLKYSYI</u>	<u>NKCSRFLSDD</u>	<u>RTEVPQLVNA</u>	<u>NQYSPCVSIV</u>	<u>PSTVWEDGDY</u>
<u>YRKQLSPLEG</u>	<u>GGWLVASGST</u>	<u>VAMTEQLQMG</u>	<u>FGITVQYGTD</u>	<u>TNSVCPKLEF</u>	<u>ANDTKIASQL</u>
<u>GNCVEYSLYG</u>	<u>VSGRGVFQNC</u>	<u>TAVGVRQORF</u>	<u>VYDAYQNLVG</u>	<u>YYSDDGNYYC</u>	<u>LRACVSPVPS</u>
<u>VIYDKETKTH</u>	<u>ATLFGSVACE</u>	<u>HISSTMSQYS</u>	<u>RSTRSMLKRR</u>	<u>DSTYGPLQTP</u>	<u>VGCVLGLVNS</u>
<u>SLFVEDCKLP</u>	<u>LGQSLCALPD</u>	<u>TPSTLTPRSV</u>	<u>RSVPGEMRLA</u>	<u>SIAFNHPIQV</u>	<u>DQLNSSYFKL</u>
<u>SIPTNFSFGV</u>	<u>TQEYIQTIIQ</u>	<u>KVTVDCKQYV</u>	<u>CNGFQKCEQL</u>	<u>LREYGQFCSK</u>	<u>INQALHGANL</u>
<u>RQDDSVRNLF</u>	<u>ASVKSSQSSP</u>	<u>IIPGFGDFN</u>	<u>LTLLEPVSIS</u>	<u>TGSRARSARAI</u>	<u>EDLLFDKVTI</u>
<u>ADPGYMQGYD</u>	<u>DCMQQPASA</u>	<u>RDLICAQYVA</u>	<u>GYKVLPLMD</u>	<u>VNMEAAYTSS</u>	<u>LLGSIAGVGV</u>
<u>TAGLSSFAAI</u>	<u>PFAQSIFYRL</u>	<u>NGVGITQQVL</u>	<u>SENQKLIANK</u>	<u>FNQALGAMQT</u>	<u>GFTTTNEAFH</u>
<u>KVQDAVNNA</u>	<u>QALSKLASEL</u>	<u>SNTFGAISAS</u>	<u>IGDIIQRLDV</u>	<u>LEQDAQIDRL</u>	<u>INGRLTTLNA</u>
<u>FVAQQLVRSE</u>	<u>SAALSAQLAK</u>	<u>DKVNECVKAQ</u>	<u>SKRSGFCGQG</u>	<u>THIVSFVVNA</u>	<u>PNGLYFMHVG</u>
<u>YYPNSHIEVV</u>	<u>SAYGLCDAAN</u>	<u>PTNCIAPVNG</u>	<u>YFIKTNNTRI</u>	<u>VDEWSYTGSS</u>	<u>FYAPEPITSL</u>
<u>NTKYVAPQVT</u>	<u>YQNISTNLPP</u>	<u>PLLGNSTGID</u>	<u>FQDELDEFFK</u>	<u>NVSTSIPNFG</u>	<u>SLTQINTLL</u>
<u>DLTYEMLSLQ</u>	<u>QVVKALNESY</u>	<u>IDLKELGNYT</u>	<u>YYNKWPWYIW</u>	<u>LGFIAGLVAL</u>	<u>ALCVFFILCC</u>
<u>TGCGTNCMGK</u>	<u>LKCNRCDRY</u>	<u>EEYDLEPHKV</u>	<u>HVH</u>		

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**Appendix Figure.** Protein sequence of MERS-CoV spike (S) protein used for PBMC stimulation. The MERS-CoV spike glycoprotein comprises 1353 aa (aa) with two domains, S1 (underlined in yellow) and S2 (underlined in blue). For camel PBMC stimulation, two peptide pools (S1 and S2), consisting of 168 overlapping peptides each, were derived from the MERS-CoV S protein sequence. Each single peptide consists of 15 aa (15-mers) overlapping in 11 aa with the following peptide.