Designing a therapeutic SARS-CoV-2 T-cell-inducing vaccine for high-risk patient groups

Hans-Georg Rammensee [1,2,3,4], Stefan Stevanović [1,2,3], Cécile Gouttefangeas [1,2,3], Sonja Heidu [1], Reinhild Klein [5], Beate Preuß [5], Juliane Sarah Walz [1,2,3,6], Annika Nelde [1,3,6], Sebastian P. Haen [1,2,7], Michael Reth [8], Jianying Yang [8], Ghazaleh Tabatabai [2,9,10], Hans Bösmüller [11], Helen Hoffmann [1], Michael Schindler *12, Oliver Planz [1], Karl-Heinz Wiesmüller [13], Markus W. Löffler [1,2,3,14,15]

Affiliations:

1. University of Tübingen, Institute for Cell Biology, Department of Immunology, Auf der Morgenstelle 15, 72076 Tübingen, Germany.
2. German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), partner site Tübingen, Tübingen, Germany.
3. University of Tübingen, Cluster of Excellence iFIT (EXC2180) “Image-Guided and Functionally Instructed Tumor Therapies”, Tübingen, Germany.
4. University of Tübingen, Cluster of Excellence CMFI (EXC2124) “Controlling Microbes to Fight Infections”, Tübingen, Germany.
5. University Hospital Tübingen, Department of Hematology Oncology, Clinical Immunology and Rheumatology, Tübingen, Germany.
6. University Hospital Tübingen, Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), Tübingen, Germany.
7. University Medical Center Hamburg-Eppendorf, Department of Oncology, Hematology and Bone Marrow Transplantation with Section of Pneumology, Martinistr. 52, 20246 Hamburg, Germany.
8. University of Freiburg, Department of Molecular Immunology, Institute of Biology III, BIOSS Centre For Biological Signaling Studies, Schänzlestr. 18, 79104 Freiburg, Germany.
9. University Hospital Tübingen, Comprehensive Cancer Center, Interdisciplinary Division of Neuro-Oncology, Center for CNS Tumors, Otfried-Müller-Str. 27, 72076 Tübingen, Germany.
10. Hertie Institute for Clinical Brain Research, Otfried-Müller-Str. 27, 72076 Tübingen, Germany.
11. University Hospital Tübingen, Institute of Pathology and Neuropathology, Liebermeisterstr. 8, 72076 Tübingen, Germany.
12. University Hospital Tübingen, Institute for Medical Virology and Epidemiology of Viral Diseases, Elfriede-Aulhorn-Str. 6, 72076 Tübingen, Germany.
13. EMC microcollections GmbH, Tübingen, Germany.
14. University Hospital Tübingen, Department of General, Visceral and Transplant Surgery, Tübingen, Germany.
15. University Hospital Tübingen, Department of Clinical Pharmacology, Tübingen, Germany.

Corresponding author: Markus W. Löffler, University of Tübingen, Institute for Cell Biology, Department of Immunology, Auf der Morgenstelle 15, 72076 Tübingen, Germany; Tel.: +49/ 7071/ 29 80992; E-mmail: markus.loeffler@uni-tuenbingen.de

Keywords: SARS-CoV-2, vaccine, peptide, adjuvant, lipopeptide, therapeutic vaccine, high-risk patient, COVID-19

Abstract

Here, we describe the preliminary results of an experimental vaccination of a self-experimenting healthy volunteer with eight SARS-CoV-2-derived peptides: five predicted to bind to HLA class I molecules (CD8 peptides) and three predicted to bind to HLA-DR molecules (CD4 peptides). The vaccine formulation also included one long and one short CMV-pp65-derived peptide that had previously been administered to the same individual and could thus act as positive controls. It further contained the new adjuvant XS15 and was administered as an emulsion in Montanide as a single subcutaneous (s.c.) injection. Peripheral blood mononuclear cells (PBMCs) isolated from blood drawn on day 36 before vaccination and day 19 after vaccination were assessed using an *ex vivo* Interferon-γ ELISpot assay. We detected strong vaccine-induced T-cell responses against all four CD4 peptides and against the recall CMV CD8 epitope, but found no immune responses against the five predicted SARS-CoV-2 CD8 peptides. Antibody reactivity against all the SARS-CoV-2 CD4 peptides, as detected using ELISA, was negative or marginal. We interpret these results in terms of the prospects of a therapeutic vaccine to be applied in symptomatic COVID-19 patients. An advantage of this approach is the possibility to assess efficacy or failure within a short time after vaccination.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes corona virus disease 2019 (COVID-19), was first sampled and described in December 2019 in Wuhan, China [1]. It has a high basic reproduction number (R_0), estimated at 3.28 [2], with person-to-person transmission even occurring between asymptomatic or presymptomatic individuals [3]. This virus has emerged as a global threat and brought many national health systems to the limit of their treatment capacities.

COVID-19 symptoms can be mild or even negligible and initial manifestations encompass non-specific flu-like symptoms, such as fever, cough and fatigue [4]. Most infected individuals will recover without medical intervention, but a relevant number of patients require medical attention due to severe or even life-threatening symptoms, such as pneumonia, acute respiratory distress syndrome and a syndrome with similar features to a cytokine storm [5]. Increased cytokine levels (including IL-6, IL-10 and TNF), lymphopenia (affecting both $CD4^+$ and $CD8^+$ T cells) and a reduced expression of IFNγ in $CD4^+$ T cells have been observed and reported to be associated with COVID-19 severity [4].

While a severe clinical manifestation of SARS-CoV-2 infection may affect any person, risk groups for disease complications mainly include individuals with (multiple) co-morbidities and the elderly, who have a disproportionally high mortality [6].

The international scientific community is working at an unprecedented pace to find therapeutic and prophylactic responses to COVID-19. This includes attempts to develop vaccines. Important strategies to pursue are: passive immunotherapies, such as plasma transfer from convalescent individuals [7,8], as practiced for other infectious diseases since 1892; and active immunotherapies, intended to treat at-risk COVID-19 patients. To the best of our knowledge, the potential of therapeutic vaccines has yet to be widely considered.

We previously reported the results of an experimental approach based on a peptide vaccine that comprises multiple peptides consisting of various viral epitopes. Peptides were administered together with Montanide and a novel lipopeptide adjuvant, the TLR2/1 ligand XS15, to the same healthy volunteer [9].

Building on this previous work, here we report the findings from self-experimentation involving the same volunteer. The previously tested protocol for vaccination was used, but this time the vaccine contained *in silico* predicted peptide sequences from a SARS-CoV-2 virus isolate. Relevant data that were available very early from a virus isolate from China were used for this purpose [10].

Experimental procedures

The sequences described here are derived from a virus isolate obtained from a worker at the Wuhan fish market (https://www.ncbi.nlm.nih.gov/nuccore/MN908947 [10]). Using the SARS-CoV-2 nucleocapsid and envelope protein sequences from this isolate and the prediction software SYFPEITHI (www.syfpeithi.de; [11]) we predicted candidate CD8 (HLA-A*01 or -B*08) and candidate CD4 T-cell epitopes (HLA-DRB1*11) based on the HLA allotype of the healthy volunteer. The resulting sequences were then run on other widely used more up to date software for confirmation. We chose the nucleocapsid protein, because nucleoprotein peptides from many other viruses are potent T-cell epitopes (see: www.iedb.org).

Peptide selection was completed on January 23, 2020. We synthesized eight peptide sequences that had the highest SYFPEITHI-scores and also appeared to be good candidates, based on our subjective experience, for the SARS-CoV-2 nucleocapsid or envelope proteins (i.e., they were predicted to be HLA ligands for the corresponding HLA molecules) and that had no cysteine residues (for chemical stability reasons). They were complemented by two CMV-derived peptides that had previously elicited immune responses in the same individual and could thus be used as positive controls [9]. These ten peptides (**Table 1**) were synthesized in-house using automated peptide synthesis.

The personalized vaccine consisted of the peptides (240 µg or 720 µg of each; see **Table 1.**) solubilized in water and 20% dimethyl sulfoxide (DMSO). The vaccine contains two out of the ten candidate peptide sequences derived from the SARS-CoV-2 nucleocapsid predicted to bind to HLA-DR, shown in **Table 2** (respective predictions were performed much later, when the idea of the potential merit of a therapeutic T-cell epitope-only vaccine had occurred to us). It further contained the lipopeptide adjuvant XS15 (50 µg) and was administered as an emulsion in Montanide™ ISA51 VG (Seppic, Paris, France). A total volume of 0.5 ml was self-administered subcutaneously (s.c.) under the skin of the abdomen on March 6, 2020. The vaccinated individual did not report any flu-like symptoms or other symptoms consistent with SARS-CoV-2 infection during the relevant period. The individual did not report having visited any areas designated as high risk for virus transmission.

Heparin-anticoagulated peripheral venous blood was drawn 36 days before and 19 days after vaccination PBMCs were isolated via density centrifugation [12] and either frozen and stored in liquid nitrogen (pre-vaccination PBMCs) or used fresh (post-vaccination PBMCs). In addition, plasma diluted 1:1 with phosphate-buffered saline (PBS) was obtained from the vaccinated volunteer during PBMC isolation. Blood serum from three healthy blood donors (HDs) was obtained after informed consent for use as control samples.

PBMCs obtained pre-vaccination were thawed and rested overnight in culture medium (IMDM with 10% heat-inactivated human serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin and 50 µM β-mercaptoethanol), containing 1 µg/ml DNase I. PBMCs obtained post-vaccination were used directly *ex vivo* after density gradient separation. In both cases, 300,000 cells per well were plated on a pre-coated ELISpot plate. The negative control (DMSO and water) and

vaccine peptides were respectively tested in six and three replicates. For phytohemagglutinin (PHA-L) stimulation (positive control), duplicates were plated with 150,000 cells per well. Peptides were added at a concentration of 5 µg/ml (for HLA class I peptides) or 2.5 µg/ml (for HLA-DR peptides), and PHA-L was used at 10 µg/ml. The interferon-γ (IFNγ) ELISpot was performed as described previously [9,12] with incubation at 37°C in a 7.5% CO_2 atmosphere for 26 h before development.

Antibody reactivity in the plasma obtained from the vaccinated volunteer and in serum from the three HDs was used in an in-house ELISA performed as described previously [12]. Briefly, 96-well plates were incubated for 16 h with the respective peptides (produced *in-house* at the University of Tübingen, Department of Immunology, Tübingen, Germany) or with recombinant proteins (SinoBiological, Peking, China), utilized at a concentration of either 35 µg/ml or 1 µg/ml. Plasma or serum samples were diluted 1:500 (previously determined to be optimal for the analysis of peptide–antibody reactivity). To differentiate between IgG- and IgM-antibodies, peroxidase-conjugated goat anti-human IgG- and IgM-antibodies were applied in parallel. Ortho-phenylene-diamine was used as a substrate. The reactivity was measured with an ELISA-reader at 450 nm and is expressed as the optical density multiplied by 1,000 (OD x 10^3). Cut off values were determined testing sera from healthy blood donors against the respective antigens/ peptides. Values above the mean of OD x 10^3 plus twice the standard deviation were defined as antibody positive.

Results

Ethical and scientific considerations

As previously reported in a comparable self-vaccination study by the same human volunteer [9], we consider this to be an ethically and legally legitimate form of experimentation [13]. Self-experimentation is a special case of research and limited to individual subjects. It is essential that such self-experimentation does not contravene any questions of personal interest or ethical imperative. Coercion and dependency can be excluded here, ensuring decision-making autonomy. The volunteer in this case is a renowned expert in immunology and thus was able to understand any risks and implications of his own actions. It should therefore be clear that this study was permissible. In addition, relevant precedent exists, where human self-experimentation has opened new avenues for research and contributed to medical progress [14].

We are naturally aware that single case reports cannot provide conclusive evidence or any generalizable results. Rather these findings enable the formulation of new hypotheses. Case reports are attributed with a high sensitivity for detecting novelty and are deemed relevant for medical progress [15]. This study is intended as a starting point for intensified discussion and development, rather than a substitute for proper drug development and clinical trials.

Clinical aspects of the vaccination site

As expected with any s.c. vaccination using Montanide [16] or when further adding XS15 as an adjuvant [9], a granuloma developed. It was palpable from day one and grew to a maximum size of approximately 3 x 5 x 1.5 cm by day 12. The skin surface temperature was 37.0°C at the center of the granuloma vs. 36.2°C on adjacent visually unaffected abdominal skin. The granuloma was described as a painless, slightly itchy induration that was sensitive to touch between days 9 and 14, and. It started to shrink in size from day 13.

T-cell responses

Ex vivo IFNγ ELISpot results from pre- and post-vaccination PBMCs are shown in **Figs 1a & b**. The CMV-pp65 HLA-DR epitope YQEFFWDANDIYRIF (amino acids 510–524), which was included as a positive control, was weakly recognized (mean: 16 spots/300,000 cells). Notably, three years prior, vaccination with this peptide resulted in a mean spot count of 525 four weeks after it had been administered, with the same adjuvant and protocol [9]. As expected from our previous experience, boosting with one additional vaccination more than one year later with the same peptide (CMV-pp65 $_{510–524}$) gave a strong response (mean: 910 spots/300,000 cells). The CMV-pp65 HLA-A*01 epitope YSEHPTFTSQY (amino acids 363–373), which had induced a weak response in an *ex vivo* ELISpot three years prior with a mean spot count of only 12, and showed a negative response before the recent booster vaccination, now gave a mean spot count of 115. These results indicate that a three-year memory against these HLA class II- and class I-restricted CMV-pp65 epitopes prevails after a single vaccination. Importantly, the vaccinated volunteer had previously been tested as CMV seronegative.

No other pre-existing T-cell responses were detectable prior to vaccination. The response for the five predicted SARS-CoV-2 CD8 peptides remained negative after vaccination. Based on previous findings, we speculate that some of these CD8 peptides may show positive results after a 12-day *in vitro* restimulation (ongoing experiment) or in *ex vivo* ELISpot with blood obtained later. We intend to test this with blood samples drawn about one month after vaccination.

By contrast, a strong T-cell response was induced against all three SARS-CoV-2 CD4 peptides by a single vaccination: a mean of 214 spots for the nucleoprotein-derived peptide ASAFFGMSRIGMEVT; 71 spots for the nucleoprotein-derived peptide IGYYRRATRRIRGGD; and 29 spots for the envelope protein-derived peptide FYVYSRVKNLNSSRV. Thus, all three SARS-CoV-2-derived CD4 peptides induced T cells to produce IFNγ, most probably representing a T_H1 response. Phenotyping of the responding T-cell subsets and the production of further cytokines will be tested using intracellular cytokine staining (ongoing experiments).

Based on these promising results, a second vaccination attempt with a new peptide cocktail designed to be suitable for all individuals independent of their HLA type (see **Table 3**) was performed by the self-vaccinating individual on April 3, 2020.

Antibody responses

The antibodies contained in the plasma of the self-experimenting volunteer and serum of three healthy blood donors were tested using an ELISA. IgG and IgM antibodies against the three SARS-CoV-2 CD4 peptides were not detectable or showed negligible induction (**Table 4**). We know from previous work that repeated vaccination with CD4 peptides in Montanide with or without additional adjuvants leads to the induction of antibodies [12]. We therefore speculate that antibodies against these epitopes may develop later on and will evaluate this with future blood samples. There seems to be a weak recall IgG response against the CMV-pp65 $_{510–524}$ CD4 epitope YQEFFWDANDIYRIF (an 8-fold increase compared to the reactivity before vaccination). It is interesting that two out of the three healthy donors show high IgG reactivity against YQEFFWDANDIYRIF. Testing serum for antibodies against linear synthetic peptides from all proteins, not only from surmised neutralizing epitope-bearing ones, might be a useful complement to the use of recombinant proteins.

In addition, sera of the vaccinated volunteer and several healthy donors, obtained before the current pandemic, were tested with an ELISA covering SARS-CoV-2 proteins and peptides (**Table 5**). Further, serum from one donor (HD CoV+-1), was included, who was tested SARS-CoV-2-positive according to medical routines (qRT-PCR) and had recovered from the infection.

Discussion

Ex vivo IFNγ-ELISpot results revealed strong T-cell responses against all three SARS-CoV-2-derived CD4 peptides 19 days after a single s.c. vaccination with the peptides, Montanide and the toll-like receptor (TLR) 1/2 ligand XS15. Based on previous findings, we assume that these immune responses are most probably robust and durable [9]. There were no detectable T-cell responses against the five CD8 peptides and no measurable antibody responses.

We are currently considering the development of a therapeutic SARS-CoV-2 peptide vaccine that would induce the same profile of immune responses seen here in one self-experimenting human volunteer. The potential of such an approach is based on the following assumptions:

1. $CD4^+$ T_H1 cells should vigorously activate virus antigen-experienced B cells that should already pre-exist in most COVID-19-patients. An illustration for this assumption is provided in **Fig. 2**. These $CD4^+$ T cells would also be expected to directly contribute to virus clearance and deliver strong T helper signals to the $CD8^+$ T cells already primed during natural infection. The resulting enhanced activity could lead to more rapid virus clearance and/or transiently increased lung damage.

2. Vaccine-induced $CD8^+$ T cells may appear later (based on our own experience with this vaccine approach), and should therefore not immediately induce or exacerbate a potential $CD8^+$ T-cell-mediated damage of lung tissue. Note that all 10 proposed vaccine peptides contain embedded CD8 candidate epitopes predicted to bind to many

HLA class I allotypes. Once activated, such $CD8^+$ T cells should also contribute to faster virus clearance, but potentially also to temporarily increased lung damage, while the virus is present, in case this emerges as an issue with CD8 T cells.

3. Vaccine-induced antibodies against the viral peptides tested in this study may also appear much later, if at all. Thus, we assume that there is no immediate danger of vaccine-induced antibody-dependent enhancement (ADE) as described for anti-spike IgG in acute SARS-CoV infections [17,18]. However, there is a danger of vaccine-induced ADE in cases where the patient' s B cells have already been primed against epitopes from the regular seasonal coronavirus strains that infect humans, produce low amounts of antibodies, antibodies with low affinity or antibodies with the wrong class. This danger should also be considered and may be causal for ADE, triggered in the course of natural infection. In theory, vaccine-induced $CD4^+$ T cells thus might also cause or exacerbate immunopathological effects indirectly.

4. Since we found IFNγ-producing T cells, it is very likely that T_H1 $CD4^+$ T cells are present. Therefore, there should be no disease enhancing-effects related to the induction of T_H2-bias as described for other corona viruses [19].

Of course, a vaccine designed for broad use must be designed to be suitable for all patients independent of their individual HLA allotypes. We propose that the ten SARS-CoV-2 nucleocapsid protein-derived peptides, carefully selected for this purpose to be promiscuous for most HLA-DR molecules (**Table 2**) should be used for a first exploration of this vaccine concept that we called CovidFort. Together, these 10 peptides should cover the vast majority of the population. Additional peptides from the spike glycoprotein or other viral proteins not expected to contain any linear antibody epitopes could be added after careful examination of the risk of inducing ADE through non-neutralizing antibodies improving viral uptake as has been described for SARS-CoV-1 and dengue virus infection in humans and feline infectious peritonitis virus in cats [20-22]. There might also be strong antibody responses (high titers) against certain spike epitopes that are neutralizing, whereas the same antibodies at low titer result in enhancing [23]. The most rigid way to circumvent this difficult issue is to completely exclude potential linear antibody epitopes from early stage for therapeutic peptide vaccines. Thus, our strategy is to carefully select spike glycoprotein-derived CD4 candidate epitopes that are promiscuous for most HLA-DR allotypes, but that do not represent any potential B-cell epitopes, as far as can be judged using *in silico* analysis and analysis of serum antibodies from SARS-CoV-2-infected individuals (ongoing investigation on a vaccine approach called CovidFort). Only later, when we and others have gained additional experience, we would dare adding spike CD4 peptides, containing linear antibody epitopes confirmed as neutralizing, or determined as antibody targets in convalescent patients recovered from SARS-Cov-2-infection, or even in seemingly healthy individuals with high titers of spike antibodies, since these individuals should have had an unnoticed SARS-CoV-2 infection that was cleared by particularly efficient immune responses. The relevant underlying theoretical considerations are illustrated in **Fig. 2**.

This strategy has the advantage of enabling quick assessment of vaccine efficacy as well as any adverse effects in terms of induction of immune responses and potential viral load reduction in SARS-CoV-2-infected patients with and without symptoms. We speculate that effects could be determined within one week after administration in the following manner:

i) The percentage of SARS-CoV-2 PCR-negative patients on day seven (or later), would demonstrate the efficacy of virus clearance compared to the results for untreated patients.

ii) The vaccine-induced increase in the levels of antibodies against SARS-CoV-2 proteins, including the spike glycoprotein, could be easily measured in short intervals after vaccination.

In addition, the Montanide-induced granuloma, usually seen as a nuisance, here enables to quick termination of most of the vaccine-induced T-cell reactivity in patients that develop increased immunopathology in the first days after vaccination. Local injection of steroids into the granuloma or even its surgical removal should broadly terminate the induced immune responses, since most of the activated immune cells are initially located inside the granuloma within newly developing lymphoid structures.

Further considerations led us to the hypothesis that vaccination with T-cell epitopes alone might also prove useful for prophylactic vaccination. As the situation in zoonotic coronaviruses suggests, this strategy might even result superior to traditional vaccination approaches, where primarily B-cell responses against the vaccine antigens are to be induced, since in many such infections mainly memory T-cells and not antibodies seem to play the major role for long term-immunity against disease [24]. A vaccine solely consisting of T-cell epitopes would not be expected to completely prevent infection, as with traditional vaccines aiming for strong neutralizing antibody responses, but to help the patient's immune system to quickly resolve the commencing infection by fostering faster antibody production, enabled by the vaccine-induced $CD4^+$ T cells. Overall, in contrast to many treatments currently introduced in COVID-19 and used on patients off-label, we expect therapeutic vaccines to have a particularly positive risk-benefit ratio.

This potential for a lack of prevention that may be seen as a disadvantage should be counterbalanced by the broader cross-reactivity of T cells, as seen in the example of influenza [25]. Thus, a T-cell epitope-based vaccine against influenza should be an efficient prophylaxis against several seasonal influenza strains, provided these strains contain the T-cell epitopes included in the vaccine (**Fig. 2**). In the case of SARS-CoV-2, this type of vaccine should also work for new viral serotypes and variants that already exist in so far unknown environments or could develop in the future.

Taken together, we propose a therapeutic immunization strategy targeting primarily T cells, but affecting B cells only indirectly, to be applied in at-risk patients after SARS-CoV-2 infection. We do not know at this time, whether such a strategy will be successful in eliminating the virus more rapidly or does comprise risks that may be harmful for the patients. An advantage of the

approach is that it can be tested **very quickly**: Vaccinating infected elderly patients (e.g. >70 years old) as soon as possible after confirmed infection may yield results within only 7 days after vaccination. Lacking effects, improvement or deterioration of symptoms, an increase in antibody responses, or rapid virus clearance should guide us in the right direction.

Acknowledgments

The authors would like to thank Patricia Hrstić, Monika Denk, Marina Martin, Ulrich Wulle and Marion Gauger at the Wirkstoffpeptidlabor of the Department of Immunology of the University of Tübingen for their invaluable support with the production of peptides and excellent technical support.

Conflicts of Interest

H.G. Rammensee has ownership interest in Immatics Biotechnologies GmbH, CureVac AG, Bamomab GmbH, and Synimmune GmbH. H.G. Rammensee and K.H. Wiesmüller share the patent for XS15. K.H. Wiesmüller further holds ownership interest in EMC microcollections GmbH.
O. Planz has ownership interest in Atriva Therapeutics GmbH and is a consultant for Atriva Therapeutics GmbH. H. Hoffmann is an employee of Atriva Therapeutics GmbH.
H.G. Rammensee, A. Nelde, J.S. Walz, S.P. Haen, S. Stevanović and M.W. Löffler are the inventors of patents for vaccine peptides owned by Immatics, but unrelated to SARS-CoV-2.
M.W. Löffler acts as a paid consultant in cancer immunology for Boehringer Ingelheim Pharma GmbH & Co. KG.
All other authors declare no potential conflicts of interest.

Funding

Generic funding was provided by the University of Tübingen, the University Hospital Tübingen as well as the DFG Cluster of Excellence "iFIT – Image-Guided and Functionally Instructed Tumor Therapies" (EXC 2180, to H.G. Rammensee, S. Stevanović, C. Gouttefangeas, J.S. Walz, A. Nelde and M.W. Löffler). Additional funding was granted to HG. Rammensee by the Ernst-Jung Stiftung and from the Medical Faculty at the University of Tübingen by an intramural grant (“Angewandte Klinische Forschung” (AKF) program to G. Tabatabai and H.G. Rammensee) with regard to GMP-manufacturing of XS15.

Gefördert durch die Deutsche Forschungsgemeinschaft (DFG) im Rahmen der Exzellenzstrategie des Bundes und der Länder - EXC 2180 (390900677).

References

1 Zhu, N. *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* **382**, 727-733, doi:10.1056/NEJMoa2001017 (2020).

2 Liu, Y., Gayle, A. A., Wilder-Smith, A. & Rocklov, J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J Travel Med* **27**, doi:10.1093/jtm/taaa021 (2020).

3 Chan, J. F. *et al.* A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* **395**, 514-523, doi:10.1016/S0140-6736(20)30154-9 (2020).

4 Chen, G. *et al.* Clinical and immunologic features in severe and moderate Coronavirus Disease 2019. *J Clin Invest*, doi:10.1172/JCI137244 (2020).

5 Pedersen, S. F. & Ho, Y. C. SARS-CoV-2: A Storm is Raging. *J Clin Invest*, doi:10.1172/JCI137647 (2020).

6 Guan, W. J. *et al.* Comorbidity and its impact on 1590 patients with Covid-19 in China: A Nationwide Analysis. *Eur Respir J*, doi:10.1183/13993003.00547-2020 (2020).

7 Casadevall, A. & Pirofski, L. A. The convalescent sera option for containing COVID-19. *J Clin Invest* **130**, 1545-1548, doi:10.1172/JCI138003 (2020).

8 Shen, C. *et al.* Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. *JAMA*, doi:10.1001/jama.2020.4783 (2020).

9 Rammensee, H. G. *et al.* A new synthetic toll-like receptor 1/2 ligand is an efficient adjuvant for peptide vaccination in a human volunteer. *J Immunother Cancer* **7**, 307, doi:10.1186/s40425-019-0796-5 (2019).

10 Wu, F. *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* **579**, 265-269, doi:10.1038/s41586-020-2008-3 (2020).

11 Rammensee, H., Bachmann, J., Emmerich, N. P., Bachor, O. A. & Stevanovic, S. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* **50**, 213-219, doi:10.1007/s002510050595 (1999).

12 Widenmeyer, M. *et al.* Promiscuous survivin peptide induces robust CD4+ T-cell responses in the majority of vaccinated cancer patients. *Int J Cancer* **131**, 140-149, doi:10.1002/ijc.26365 (2012).

13 Hanley, B. P., Bains, W. & Church, G. Review of Scientific Self-Experimentation: Ethics History, Regulation, Scenarios, and Views Among Ethics Committees and Prominent Scientists. *Rejuvenation Res* **22**, 31-42, doi:10.1089/rej.2018.2059 (2019).

14 West, J. B. The beginnings of cardiac catheterization and the resulting impact on pulmonary medicine. *Am J Physiol Lung Cell Mol Physiol* **313**, L651-L658, doi:10.1152/ajplung.00133.2017 (2017).

15 Vandenbroucke, J. P. In defense of case reports and case series. *Ann Intern Med* **134**, 330-334, doi:10.7326/0003-4819-134-4-200102200-00017 (2001).

16 Löffler, M. W. *et al.* Personalized peptide vaccine-induced immune response associated with long-term survival of a metastatic cholangiocarcinoma patient. *J Hepatol* **65**, 849-855, doi:10.1016/j.jhep.2016.06.027 (2016).

17 Liu, L. *et al.* Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. *JCI Insight* **4**, doi:10.1172/jci.insight.123158 (2019).

18 Peeples, L. News Feature: Avoiding pitfalls in the pursuit of a COVID-19 vaccine. *Proc Natl Acad Sci U S A*, doi:10.1073/pnas.2005456117 (2020).

19 Honda-Okubo, Y. *et al.* Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology. *J Virol* **89**, 2995-3007, doi:10.1128/JVI.02980-14 (2015).

20 Dejnirattisai, W. *et al.* Cross-reacting antibodies enhance dengue virus infection in humans. *Science* **328**, 745-748, doi:10.1126/science.1185181 (2010).

21 Takano, T., Kawakami, C., Yamada, S., Satoh, R. & Hohdatsu, T. Antibody-dependent enhancement occurs upon re-infection with the identical serotype virus in feline infectious peritonitis virus infection. *J Vet Med Sci* **70**, 1315-1321, doi:10.1292/jvms.70.1315 (2008).
22 Yang, Z. Y. *et al.* Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. *Proc Natl Acad Sci U S A* **102**, 797-801, doi:10.1073/pnas.0409065102 (2005).
23 Tetro, J. A. Is COVID-19 receiving ADE from other coronaviruses? *Microbes Infect* **22**, 72-73, doi:10.1016/j.micinf.2020.02.006 (2020).
24 Channappanavar, R., Zhao, J. & Perlman, S. T cell-mediated immune response to respiratory coronaviruses. *Immunol Res* **59**, 118-128, doi:10.1007/s12026-014-8534-z (2014).
25 Markovic-Plese, S. *et al.* High level of cross-reactivity in influenza virus hemagglutinin-specific CD4+ T-cell response: implications for the initiation of autoimmune response in multiple sclerosis. *J Neuroimmunol* **169**, 31-38, doi:10.1016/j.jneuroim.2005.07.014 (2005).
26 Avalos, A. M. & Ploegh, H. L. Early BCR Events and Antigen Capture, Processing, and Loading on MHC Class II on B Cells. *Front Immunol* **5**, 92, doi:10.3389/fimmu.2014.00092 (2014).
27 Serangeli, C. *et al.* Ex vivo detection of adenovirus specific CD4+ T-cell responses to HLA-DR-epitopes of the Hexon protein show a contracted specificity of T(HELPER) cells following stem cell transplantation. *Virology* **397**, 277-284, doi:10.1016/j.virol.2009.10.049 (2010).

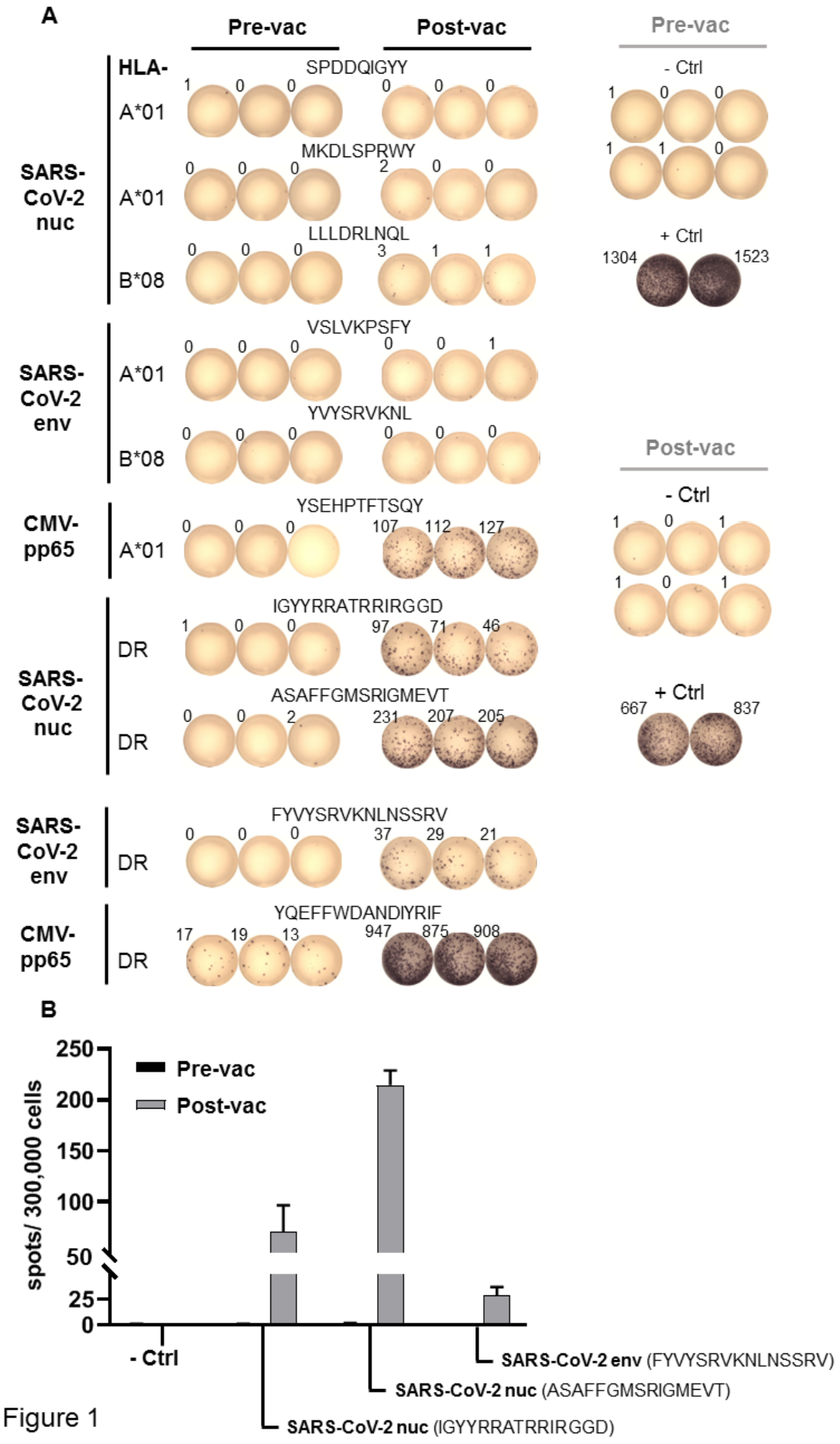
A
Pre-vac
Post-vac
HLA-
SARS-CoV-2 nuc
A*01
SPDDQIGYY
1 0 0 0 0 0
MKDLSPRWY
0 0 0 2 0 0
B*08
LLLDRLNQL
0 0 0 3 1 1
SARS-CoV-2 env
VSLVKPSFY
0 0 0 0 0 1
YVYSRVKNL
0 0 0 0 0 0
CMV-pp65
YSEHPTFTSQY
0 0 0 107 112 127
DR
IGYYRRATRRIRGGD
1 0 0 97 71 46
ASAFFGMSRIGMEVT
0 0 2 231 207 205
FYVYSRVKNLNSSRV
0 0 0 37 29 21
YQEFFWDANDIYRIF
17 19 13 947 875 908
Pre-vac
- Ctrl
1 0 0
1 1 0
+ Ctrl
1304 1523
Post-vac
- Ctrl
1 0 1
1 0 1
+ Ctrl
667 837
B
spots/ 300,000 cells
250
200
150
100
50
25
0
Pre-vac
Post-vac
- Ctrl
SARS-CoV-2 nuc (IGYYRRATRRIRGGD)
SARS-CoV-2 nuc (ASAFFGMSRIGMEVT)
SARS-CoV-2 env (FYVYSRVKNLNSSRV)

Figure 1

Figure legends

Fig. 1. Results of the *ex vivo* Interferon-γ ELISpot.

(A) ELISpot wells showing spot counts obtained from pre- and post-vaccination PBMC samples tested against each of the specified vaccine peptides (n = 10, in triplicate). The negative control (- Ctrl: DMSO and water) was tested in six replicate wells and the positive control (+ Ctrl: phytohemagglutinin) in duplicate wells. The images representing the ELISpot wells were rearranged for this illustration. **(B)** Graph of spot numbers for the three SARS-CoV-2-derived HLA-DR peptides. The mean and SD are shown. Abbreviations: CMV: cytomegalovirus, Ctrl: control, DMSO: dimethyl sulfoxide, env: envelope protein, nuc: nucleoprotein, pre-vac: 36 days before vaccination, post-vac: 19 days after vaccination, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

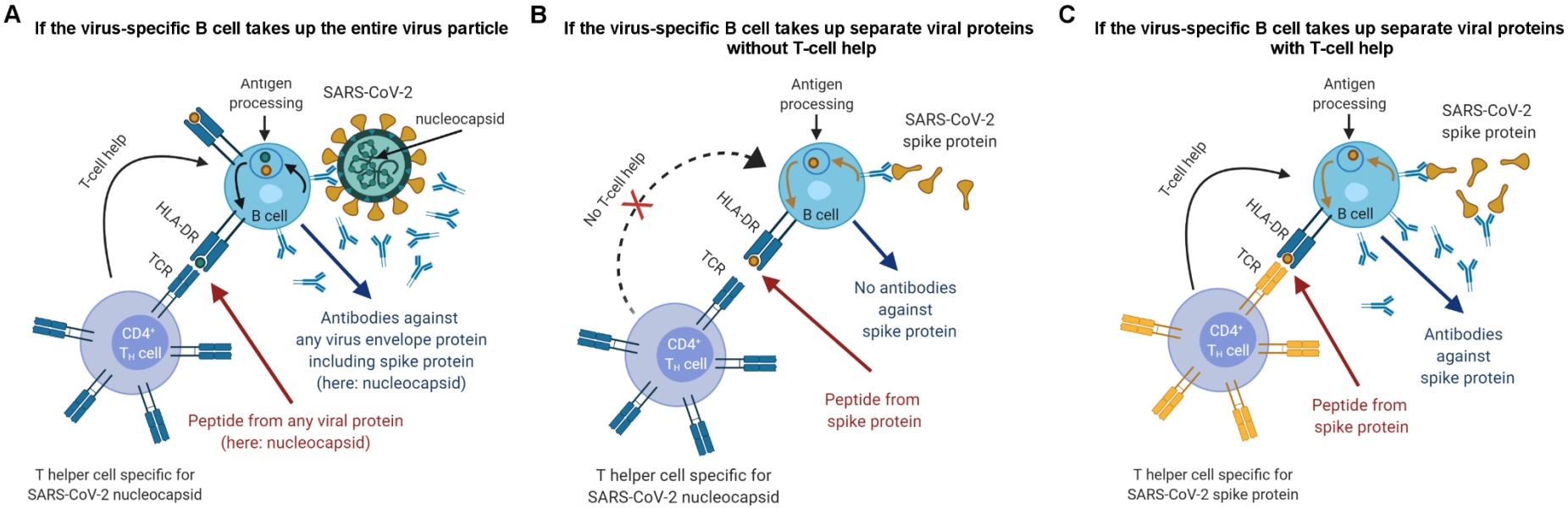

Fig. 2. Illustration of the potential interactions between the virus and B and T cells.

(A) If a virus-specific B cell takes up the entire virus particle via B cell receptor-mediated (BCR-mediated) phagocytosis, all the structural viral proteins should be processed in the HLA class II processing vesicle. The resulting peptides should be loaded on HLA class II molecules. For a review, see Avalos et al. (2014)[26]. Only a selection of the resulting peptides will fit to the respective HLA molecules present based on their peptide specificity. The B cell will then present these peptides on the cell surface, with one peptide per HLA molecule. If a T cell is specific for exactly this peptide–HLA combination **and** if the B cell has been activated via its BCR–antigen contact, then the $CD4^+$ T helper cell will deliver help to this B cell, both trough cellular interaction and cytokines. Since all viral proteins are presented on the B cell's HLA in this scenario, a nucleocapsid-specific T cell will also activate a spike-specific B cell. **(B)** If the virus-specific B cell takes up separate viral proteins via BCR-mediated phagocytosis, e.g., after previous destruction of viral particles by follicular dendritic cells, only peptides from these proteins will be presented on the B cell's HLA molecules. This is shown here for the spike glycoprotein. Thus, a nucleocapsid-specific T cell will not activate a spike-specific B cell in this constellation. **(C)** If the same B cells as described in B) are activated by a spike-specific $CD4^+$ T helper cell, the B cell will now be activated. This constellation is described in the last part of the discussion. Simply replace "SARS-CoV-2 spike protein" with, e.g., "Influenza haemagglutinin" to make this congruent for the case of influenza infection. Abbreviations: HLA: human leucocyte antigen, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, TCR T-cell receptor.

Table 1. Peptides included in the vaccine self-administered by one healthy volunteer (first vaccination).

Vaccination was performed with synthetic peptides solubilized in water and 20% DMSO including 50 µg XS15 as an adjuvant, emulsified in Montanide ISA51 VG in a total volume of 0.5 ml. The varying dosing of peptides (240 µg for all HLA-class II restricted peptides and 720 µg for some of the HLA-class-I restricted peptides) was used to address technical questions, based on speculations that a higher antigen load might induce better CD8 T cell responses for HLA-class I-restricted peptides in this particular vaccine approach. Abbreviations: AA: amino acid, DMSO: dimethyl sulfoxide, env: envelope protein, HLA: human leukocyte antigen, nuc: nucleoprotein.

AA sequence	Predicted HLA restriction	Source protein	Peptide ID	AA position	Peptide length	Administered amount (µg)
SPDDQIGYY	A*01	SARS-CoV-2 nuc	Nuc-A1-1	79–87	9	240
MKDLSPRWY	A*01		Nuc-A1-2	101–109	9	
LLLDRLNQL	B*08		Nuc-B1-1	222–230	9	
IGYYRRATRRIRGGD	DR		Nuc-DR-1	84–98	15	
ASAFFGMSRIGMEVT	DR		Nuc-DR-2	311–325	15	
VSLVKPSFY	A*01	SARS-CoV-2 env	Env-A1-1	49–57	9	720
YVYSRVKNL	B*08		Env-B8-1	57–65	9	720
FYVYSRVKNLNSSRV	DR		Env-DR-1	56–70	15	240
YSEHPTFTSQY	A*01	CMV-pp65	CMV-A1-1	363–373	11	720
YQEFFWDANDIYRIF	DR		CMV-DR-1	510–524	15	240

Table 2. Selection of peptides from SARS-CoV-2 nucleocapsid predicted to bind to multiple HLA-DR molecules.

The genetic sequence known from a virus isolate obtained from a worker at the Wuhan fish market (https://www.ncbi.nlm.nih.gov/nuccore/MN908947 [10]), was used to predict promiscuous 15mer HLA-DR-binding candidate antigens from the SARS-CoV-2 nucleoprotein. Promiscuous means here that each of the sequences is predicted to bind to several different HLA-DR allotypes, as predicted with our in-house method described earlier [27]. The peptides included in the first vaccine provided in **Table 1** are given in bold, those used for the second vaccine are given in italics. Note: Sequences of the currently prevailing SARS-CoV-2 isolates and reported variants, need to be carefully re-evaluated before use in vaccine development. Abbreviations: aa: amino acid, HLA: human leukocyte antigen, nuc: nucleoprotein.

aa sequence	Predicted HLA restriction	Source protein	Peptide ID	aa position
IGYYRRATRRIRGGD	DR	SARS-CoV-2 nuc	Nuc-DR-1	83–98
ASAFFGMSRIGMEVT			Nuc-DR-2	311–326
KDGIIWVATEGALNT			Nuc-DR-3	128–143
GTWLTYTGAIKLDDK			Nuc-DR-4	328–343
RWYFYYLGTGPEAGL			Nuc-DR-5	107–122
ASWFTALTQHGKEDL			Nuc-DR-6	50–65
LLLLDRLNQLESKMS			Nuc-DR-7	221–236
AADLDDFSKQLQQSM			Nuc-DR-8	397–412
AIVLQLPQGTTLPKG			Nuc-DR-9	156–171
YKHWPQIAQFAPSAS			Nuc-DR-10	298–313

Table 3. Selection of peptides from SARS-CoV-2 spike glycoprotein and nucleocapsid proteins predicted to bind to multiple HLA-DR molecules included in the vaccine self-administered by one healthy volunteer (second vaccination).

Vaccination was performed with synthetic peptides solubilized in water and 20% DMSO including 50 µg XS15 as an adjuvant, emulsified in Montanide ISA51 VG in a total volume of 0.5 ml. The amount of peptides administered in the vaccine was 240 µg for each peptide.

Abbreviations: aa: amino acid, DMSO: dimethyl sulfoxide, HLA: human leukocyte antigen, nuc: nucleoprotein.

aa sequence	Predicted HLA-restriction	Source protein	Peptide ID	aa position	Peptide length
ASVYAWNRKRISN	DR	SARS-CoV-2 spike	Spi-DR-1	348–360	13
VADYSVLYNSASFST			Spi-DR-2	362–376	15
IGYYRRATRRIRGGD		SARS-CoV-2 nuc	Nuc-DR-1	83–98	
ASAFFGMSRIGMEVT			Nuc-DR-2	311–326	
RWYFYYLGTGPEAGL			Nuc-DR-5	107–122	
ASWFTALTQHGKEDL			Nuc-DR-6	50–65	
LLLLDRLNQLESKMS			Nuc-DR-7	221–236	
AADLDDFSKQLQQSM			Nuc-DR-8	397–412	
AIVLQLPQGTTLPKG			Nuc-DR-9	156–171	
YKHWPQIAQFAPSAS			Nuc-DR-10	298–313	

Table 4. Antibody responses in a healthy self-vaccinated volunteer and healthy blood donors without anamnestic SARS-CoV-2 exposure.

ELISA was performed against the respective SARS-CoV-2 and CMV-pp65 using plasma samples from one vaccinated volunteer (VV) obtained 36 days before vaccine administration (pre-vac) and 19 days after vaccination with the peptides shown in **Table 1** (post-vac) and serum samples from three healthy blood donors (HD). Final dilutions of all samples were 1:500 with peptide concentrations used at 35 µg/ml. Values consdidered as positive are indicated in bold. Abbreviations: env: envelope protein, HLA: human leukocyte antigen, nuc: nucleoprotein, OD: optical density, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

	SARS-CoV-2 env		**SARS-CoV-2 nuc**		**SARS-CoV-2 nuc**		**CMV-pp65 $_{510–524}$**		**CMV-pp65 $_{363–373}$**	
HLA-	DR		DR		DR		A*01		DR	
Peptide ID	Env-DR-1		Nuc-DR-1		Nuc-DR-2		CMV-A1-1		CMV-DR-1	
	OD x 10^3									
Donor	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
VV (pre-vac)	19	41	6	13	21	13	6	10	32	49
VV (post-vac)	12	44	23	12	43	17	20	12	247	58
HD1	64	128	68	68	49	96	115	60	518	156
HD2	77	169	112	36	74	146	405	49	**1550**	146
HD3	30	441	16	67	25	69	383	63	**1457**	149

Table 5. Antibody responses in a healthy self-vaccinated volunteer and healthy blood donors with and out confirmed SARS-CoV-2 exposure.

ELISA was performed against the respective SARS-CoV-2 proteins or peptides using plasma samples from one vaccinated volunteer (VV) obtained at given time points before (pre) or after vaccination with the peptides shown in **Table 1**, as well as serum samples from healthy blood donors (HD) either confirmed as SARS-CoV-2-positive in medical routine diagnostics (qRT-PCR) or obtained before the pandemic (pre). Recombinant proteins were obtained from SinoBiologicals, for peptides used Peptide IDs are given as outlined above. Final dilutions of all samples were 1:500 with peptide concentrations used at 1 µg/ml. Values consdidered as positive are indicated in bold. Peptide sequences not mentioned elsewhere: Spi-DR-3 (SARS-CoV-2, spike glycoprotein; aa: 446–460): GGNYNYLYRLFRKSN, Spi-DR-5 (SARS-CoV-2, spike glycoprotein; aa: 458–472): KSNLKPFERDISTEI, Spi-DR-6 (SARS-CoV-2, spike glycoprotein; aa: 338–352): FGEVFNATRFASVYA. Abbreviations: RBD: receptor-binding domain, rec.: recombinant, nuc.: nucleoprotein, OD: optical density, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

	Days post Vaccination	**Ig**	**rec. RBD Protein**	**rec. Spike-1 Protein**	**rec. nuc. Protein**	**Spi-DR-1**	**Spi-DR-2**	**Spi-DR-3**	**Spi-DR-5**	**Spi-DR-6**	**Nuc-DR-3**	**Nuc-DR-9**
					OD x 10^3							
VV	pre	M	68	31	73	65	71	40	45	81	37	41
		G	96	53	59	62	76	43	39	46	45	35
VV	19	M	70	34	57	68	71	42	41	74	35	41
		G	94	47	52	62	70	43	41	34	40	31
VV	31	M	55	23	59	63	59	41	43	75	36	40
		G	80	49	56	83	63	37	31	28	31	29
HD CoV+-1	n.a.	M	**1645**	**1614**	283	213	103	108	104	177	43	180
		G	**2410**	**2586**	**2588**	176	199	100	115	115	127	125
HD-pre-1	n.a.	M	236	132	262	605	259	273	211	145	183	270
		G	347	121	**1944**	106	226	67	110	164	127	121
HD-pre-2	n.a.	M	142	94	167	452	471	316	211	226	228	96
		G	322	106	245	100	191	80	96	105	90	118
HD-pre-3	n.a.	M	289	205	385	277	324	148	255	171	179	189
		G	103	56	79	61	130	59	50	61	65	61

HD-pre-4	n.a.	M	112	80	165	265	333	237	134	108	153	89
		G	494	151	274	131	325	105	180	221	193	226
HD-pre-5	n.a.	M	366	250	498	759	385	509	425	228	297	211
		G	308	131	204	110	286	79	136	189	160	173
HD-pre-6	n.a.	M	359	293	495	394	895	288	329	272	279	365
		G	490	306	468	227	425	168	281	260	485	281