Immune Response to COVID-19 Vaccination in Elite Athletes

Lea Halmans (s8lehalm@stud.uni-saarland.de)[1], Andreas Venhorst (andreas.venhorst@uni-saarland.de)[1], Verena Klemis (verena.klemis@uks.eu)[2], Tina Schmidt (tina.schmidt@uks.eu)[2], Franziska Greiß (franziska.greiss@charite.de)[3], Urban Sester (u.sester@vk.shg-kliniken.de)[4], Barbara C. Gärtner (barbara.gaertner@uks.eu)[5], Martina Sester (martina.sester@uks.eu)[2], Tim Meyer (tim.meyer@mx.uni-saarland.de)[1]

[1] Saarland University, Department of Sports Medicine, Saarbrücken, Germany
[2]S aarland University, Department of Transplant and Infection Immunology, Homburg, Germany
[3] Department of Sports Medicine, Humboldt University and Charité, Berlin, Germany
[4] Department of Nephrology, SHG-Klinikum Völklingen, Völklingen, Germany
[5] Saarland University, Department of Medical Microbiology and Hygiene, Homburg, Germany.

ABSTRACT

Purpose: This study analyses the immune response of elite athletes after COVID-19 vaccination with double-dose mRNA and a single-dose vector vaccine.

Methods: Immunoglobulin G (IgG) antibody titers, neutralizing activity, CD4 and CD8 T-cells were examined in blood samples from 72 athletes before and after vaccination against COVID-19 (56 mRNA (BNT162b2 / mRNA-1273), 16 vector (Ad26.COV.2) vaccines). Side effects and training time loss was also recorded.

Results: Induction of IgG antibodies (mRNA: 5702 BAU/ml; 4343 BAU/ml (hereafter: median), vector: 61 BAU/ml; 52 BAU/ml, $p<0.01$), their neutralizing activity (99.7%; 10.6%, $p<0.01$), and SARS-CoV-2 spike-specific CD4 T-cells (0.13%; 0.05%; $p<0.01$) after mRNA double-dose vaccines was significantly more pronounced than after a single-dose vector vaccine. SARS-CoV-2 spike-specific CD8 T-cell levels after a vector vaccine (0.15%) were significantly higher than after mRNA vaccines (0.02%; $p<0.01$). When athletes who had initially received the vector vaccine were boostered with an mRNA vaccine, IgG antibodies (to 3456 BAU/ml; $p<0.01$), neutralizing activity (to 100%; $p<0.01$), CD4 (to 0.13%; $p<0.01$) and CD8 T-cells (to 0.43%; $p<0.01$) significantly increased. When compared with dual-dose mRNA regimen, IgG antibody response was lower ($p<0.01$), the neutralizing activity ($p<0.01$) and CD8 T-cell ($p<0.01$) response higher and no significant difference in CD4 T-cell response ($p=0.54$) between the two regimens. Cumulative training loss (3 days) did not significantly differ between vaccination regimens ($p=0.46$).

Conclusion: mRNA and vector vaccines against SARS-CoV-2 appear to induce different patterns of immune response in athletes. Lower immune induction after a single-shot vector vaccine was clearly optimized by a heterologous booster. Vaccine reactions were mild and short-lived.

**Correspondence to:*
Lea Halmans
Institute of Sports and Preventive Medicine
Saarland University
Campus, Bldg. B8 2 66123 Saarbrücken
Tel.: +49 681 302 70400
s8lehalm@stud.uni-saarland.de

INTRODUCTION

The world-wide coronavirus pandemic led to many medical, social, and health care system challenges. An infection with SARS-CoV-2 can cause severe COVID-19 with pathology including pulmonary inflammation, pulmonary fibrosis, or vascular thrombosis (1). Moreover, neurologic complications (2), olfactory and gustatory dysfunctions (3), and cardiac manifestations like myocarditis(4) may result. Important preventative/hygiene measures like frequent disinfection, wearing face masks and social distancing were recommended and used in the beginning of the pandemic(5) while different types of vaccines (vector-vaccine, mRNA vaccine, protein-based) were developed with some delay knowing that vaccinations are one of the most effective means to prevent the spread and severe courses of many infection diseases(6).

Due to vaccine shortage, it was initially necessary to prioritize older people, medical staff and other high-risk populations for vaccinations, mostly without individual choice of vaccine type. In May 2021, during the last preparation stages for the Olympic and Paralympic Games in Tokyo, aspirants for the German Olympic and Paralympic team were prioritized for vaccination based on a political decision of the German government, considering that vaccinating athletes against COVID-19 had been strongly advised (7). With different vaccine types available (and very little experience with mRNA vaccines in general), their immunogenicity and reactogenicity could be expected to differ and potentially differ in their impact on the training (e.g. time loss due to vaccine reactions) and the safety of athletes (e.g. protection from acquiring an infection) prior to and during the Olympic Games. The double-dose mRNA vaccines BNT162b2 (Comirnaty® by BioNTech) and mRNA-1273 (Spikevax® by Moderna) are based on non-replicating mRNA delivered via lipid-based nanoparticles. SARS-CoV-2 spike-encoding mRNA are translated by muscle cells or tissue resident antigen-presenting cells followed by its secretion and/or presentation on the cell surface. These viral spike proteins are recognised as foreign antigens and trigger cellular and humoral immune response(8). The mRNA vaccines were approved based on pivotal trials showing vaccination efficacy of 95%(9) and 94%(10), respectively. Overall, vaccine reactions were reported to be mild and short-lived (mean of 2-3 days) in these investigations (9, 10). The single-dose vector vaccine Ad26.COV.2 (Janssen® by Johnson&Johnson (renamed in 2022 as Jcovden®) is a recombinant, replication-incompetent human adenovirus type 26-based vector that encodes the SARS-CoV-2 spike protein, inducing expression and an immune response. It was officially approved with an effectiveness of 67% in the pivotal trial (11). At the time of the first athlete prioritization, only BNT162b2, mRNA-1273, and Ad26.COV.2 were available. It must be noted that at this time the double-dose ChAdOx1 nCoV-19 vector vaccine (by AstraZeneca) was no longer recommended for people under 60 years of age in Germany (12). Despite the considerably lower effectiveness of Ad26.COV.2 as demonstrated in the registration studies, Ad26.COV.2 was considered a practical choice for members of the German Olympic team in summer 2021 in Germany (7). A single-shot vaccination was considered promising by many athletes (and medical advisors) due to a potential induction of less vaccine side effects and possibly a faster build-up of SARS-CoV-2-specific immunity. The aspect of formally receiving a vaccinated state (meaning a certificate needed for traveling) more quickly added to the positive image of the vector vaccine particularly in the athletes.

Understanding that vaccinating athletes against SARS-CoV-2 is important, it also needs to be mentioned that sport may lead to changes in the immune system of athletes. Intensive training programs in the preparation phase for major competitions may result in an increased susceptibility to infections due to a reduction in the number of immune cells and an associated reduction in functionality(13). Therefore, it is important to understand the influence of COVID-19 vaccines on the immune system of athletes. In general data about vaccinating athletes is limited due to concerns in athletes about safety and efficacy of vaccinations - but it is important to understand more about the immune system of athletes (4, 14).

The aim of this study was to determine the immune response of elite athletes after COVID-19 vaccination as well as comparing the humoral und cellular immune response between double-dose mRNA vaccines and a single-dose vector vaccine in this population. We hypothesized a significant induction of the immune response after both vaccine types with a stronger induction of the immune response after double dose regimen compared to a single dose vector vaccine. We further hypothesized that vaccine related adverse events will overall be mild and short-lived but that training restrictions will be lower after a single dose compared to a double dose vaccine. Later changes in official vaccination policies putting more emphasis on booster vaccinations enabled us to carry out some comparison between homologous and heterologous booster vaccination in our elite athlete population.

METHODS

Participants

72 healthy elite athletes older than 16 years participated in this prospective study. Among individuals who were vaccinated with an mRNA vaccine (mean of 21 years ± 6 years (standard deviation)), 29 were females (28: BNT162b2, 1: mRNA-1273) and 27 were males (25: BNT162b2, 2: mRNA-1273). The mean age of the 5 female and 11 male athletes of the Ad26.COV.2 group was 28±5 years (standard deviation). In their respective sports discipline, the athletes performed on international or national level. Recruitment was supported by the Olympic Training Centre Saarbrücken, the University Hospital Charité Berlin and the Institute of Applied Training Science (IAT) in Leipzig mainly via personal communication with the athletes from May 2021 to September 2021. Exclusion criteria were hypersensitivity or allergy to one of the ingredients of the vaccines, a clinically relevant immunodeficiency, or an acute illness. Medication intake was not verified by means of blood profiling, but participants were explicitly asked about serious illnesses and possible treatments.

Ethics approval.

The study was carried out in accordance with the Helsinki

declaration and approved by the local ethics committee (133/21, Ärztekammer des Saarlandes, Saarbrücken, Germany). All participants were informed about the study procedures, prior to giving written informed consent. Parents signed informed consent for participants under the age of 18 years.

Study design

All participants received one out of three approved and vaccine regimens recommended at the time of the study. The regimen was chosen depending upon availability or personal preference, as a randomized controlled assignment of the vaccine was not intended and not possible under the circumstances in mid 2021. The available vaccines were mRNA-1273 (Spikevax® by Moderna, 3 athletes), BNT162b2 (Comirnaty® by BioNTech/Pfizer, 53 athletes) and Ad26.COV.2 (Jcovden® by Janssen, 16 athletes). mRNA-1273 and BNT162b2 are double-dose mRNA vaccines whereas Ad26.COV.2 was approved as a single-dose vector vaccine. Blood samples were taken before vaccination to determine baseline reactivity and exclude previous contact with SARS-CoV-2 antigens during asymptomatic infection. Moreover, short-term immunogenicity was analysed two weeks after the second dose in case of mRNA vaccines, and three weeks after the single dose vector vaccine (due to known differences in vaccine-induced peak immune responses after the first and the second vaccination(15)). Follow-up analyses were performed 6 months after the last vaccination. Further evidence for prior infection with SARS-CoV-2 was tested using an NCAP-ELISA that was performed at least once (primarily after second mRNA vaccination, or after the first Ad26.COV.2 vaccination to test for the presence of antibodies to the SARS-CoV-2 nucleocapsid protein). The study design is illustrated in figure 1. The athletes recorded all local and systemic adverse events such as pain, redness and swelling at the injection site as well as headache, fatigue, muscle pain, chills, and nausea during the first week after each vaccination by completing a standardized questionnaire. Each adverse event was rated by means of four different levels of severity. Experiencing no side effects was rated 0, whereas mild, moderate, or severe side effects were graded with 1, 2, and 3, respectively. Mild side effects were defined as adverse reactions that did not interfere with training and daily routine, moderate side effects impaired but still allowed training and daily routine, whereas severe side effects prevented training and daily routine for at least one day. Therefore, training restrictions in the context of this study were solely based on occurrence of moderate or severe side effects, whereas restrictions based on precaution were not considered. For regimens with two vaccination time points, all days with training restrictions were added to determine the total number of days lost.

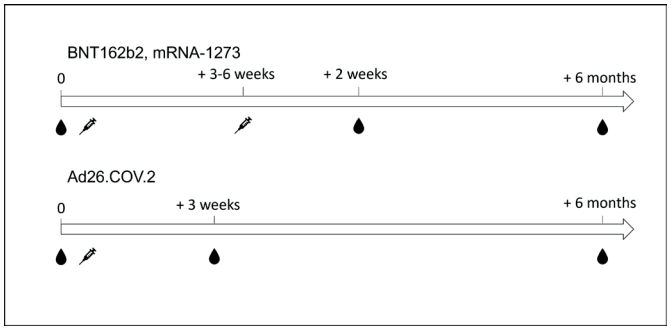

Figure 1. Overview of the study design with the vaccine regimens and their matching blood samples.

Necessary adjustments during the course of the study

After collecting the samples 2/3 weeks after vaccination and analysing the humoral and cellular immune response we found that the single-dose vector vaccine led to an insufficient humoral immune response in our athletes (e. g. median IgG antibodies after double-dose mRNA vaccination: 5702 BAU/ml, median IgG antibodies after single-dose vector vaccination: 61 BAU/ml). To provide adequate protection from COVID-19, recommendations for athletes were modified (and the study design had to be adjusted accordingly) by offering a heterologous boost vaccination to optimize the immune response in these athletes. This was carried out in 11 out of 16 athletes with the BNT162b2 vaccine after a median time of 119 days. An additional blood sample was taken two weeks after the heterologous boost to analyse the immune response. The adjusted study design can be seen in figure 2. The study adjustment was approved by the local ethics committee on September 6, 2021.

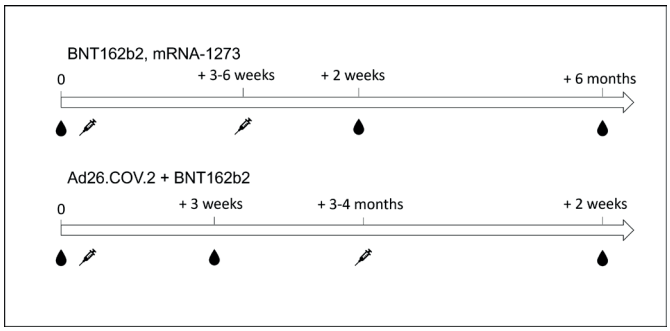

Figure 2. Overview of the adjusted study design with timelines for vaccination and blood sampling.

Procedures for immunological analyses

Lymphocyte subpopulations as well as vaccine-induced IgG antibody titers, neutralizing activity, and CD4 and CD8 T-cells were analysed from heparinized blood as previously described(16). Blood samples (9ml) were taken from an antecubital vein. The time of day was variable and deemed acceptable for our targeted parameters.

Vaccine-induced humoral immune responses were tested using ELISA assays as described by the manufacturer´s instruction (Euroimmun, Lübeck, Germany). An enzyme-linked immunosorbent assay (ELISA, SARS-CoV-2-QuantiVac) was used to quantify SARS-CoV-2 specific IgG antibodies against the receptor binding domain. Thresholds were set at <25.2 BAU/ml for being negative, ≥25.2 to <35.2 BAU/ml for being intermediate and ≥35.2 BAU/ml for being positive. An anti-SARS-CoV-2-NCP-ELISA was used to quantify SARS-CoV-2 specific IgG towards the nucleocapsid (N) protein. A surrogate neutralization assay that is based on antibody-mediated inhibition of soluble ACE2 binding to the plate bound S1 receptor binding domain (SARS-CoV-2-NeutraLISA) was used at a single serum dilution. Surrogate neutralizing capacity was calculated as percentage of inhibition (IH) by 1 minus the ratio of the extinction of the respective sample and the extinction of the blank value (16). The stimulus threshold was set according to manufacturer instructions with IH being negative under 20%, intermediate between 20 and 35% and positive over 35 %.

The protocol for quantification of SARS-CoV-2 spike-specific CD4 and CD8 T cells has been described before

(16). In brief, spike-19 specific CD4 and CD8 T-cells were quantified after a 6h stimulation with SARS-CoV-2 spike-derived overlapping peptides (each peptide 2 µg/ml, JPT, Berlin, Germany). Stimulation with 0.64% dimethyl sulfoxide (DMSO) and with 2.5 µg/ml of Staphylococcus aureus Enterotoxin B was used as a negative and positive control, respectively, to secure the specificity of the stimulation. Immunostaining was performed using anti-CD4 (clone SK3, 1:33.3), anti-CD8 (clone SK1, 1:12.5), anti-CD69 (clone L78, 1:33.3) and anti-IFNγ clone 4S.B3, 1:100, all antibodies from BD), and analyzed using flow-cytometry (BD FACS Canto II including BD FACSDiva software 6.1.3) (16). SARS-CoV-2-reactive CD4 or CD8 T-cells were identified as activated CD69-positive T-cells producing IFNγ. The percentage of specific T-cells was quantified by subtracting the percentage of T-cells after negative control stimulation from that after spike-specific stimulation. Detection limit was set at 0.03% as described before (16, 17).

Statistics

Statistical analysis was performed using R studio (version 4.0.5). Normal distribution of data was assessed using the Shapiro-Wilk test. No target parameter was distributed normally. Consequently, the nonparametric Wilcoxon test was used to analyse the quantitative parameters IgG antibody titre, neutralizing activity, CD4 and CD8 T-cells before and after vaccination. The Mann-Whitney-U-test was used to compare the immune response of the different vaccines and to analyse the vaccine side effects. The significance level was set at $p < 0.05$ for the α error. The effect size r for the Mann-Whitney-U and the Wilcoxon test was calculated with $|Z|/\sqrt{n}$ with Z being the standardised value and n the number of cases. Z was calculated with $x - \mu / \delta$. The effect size r is defined with r being small >0.10, medium >0.30 and large >0.50. No sample size analysis was performed because targeting a specific effect was not possible and intended; no comparable studies were available at that time.

RESULTS

Comparison of the immune response before and after vaccination

None of the athletes were tested NCAP-positive, which excluded a history of SARS-CoV-2 infection. The mRNA vaccines induced a significant immune response as indicated by an increase in IgG antibodies ($z=-6.5$, $p<0.01$, $r=0.87$), neutralizing antibodies ($z=-6.5$, $p<0.01$, $r=0.87$), as well as spike protein-specific CD4 ($z=-6.5$, $p<0.01$, $r=0.87$) and specific CD8 T-cells ($z=-4.9$, $p<0.01$, $r=0.70$). The aforementioned mRNA group comprises two vaccines, with mRNA-1273 being obtained from only three athletes. The IgG antibodies and neutralizing antibodies of the three athletes fall within the interquartile range of the mRNA group – which they belong to. Nevertheless, the values of the CD4 and CD8 T cells exhibit slight discrepancies, and thus, they are presented separately here (CD4 T cells: 0.05%, 0.48%, 0.67%; CD8 T cells: 0.01%, 0.05%, 0.11%). The Ad26.COV.2 vaccine also induced a significant increase in IgG antibodies ($z=-4.2$, $p<0.01$, $r=0.88$), neutralizing activity ($z=-4.2$, $p<0.01$, $r=0.88$), CD4 spike T-cells ($z=-3.4$, $p<0.01$, $r=0.87$) and CD8 spike T-cells ($z=-4.2$, $p<0.01$, $r=0.88$). Data are shown in table 1.

Comparison of the short-term immune response between the different vaccine regimens

When comparing immune-responses after vaccination, median IgG-levels were significantly higher after the mRNA vaccination ($z=-6.1$, $p<0.01$, $r=0.71$) than after the Ad26.COV.2 vaccination. This also held true for median neutralizing activity ($z=-6.1$, $p<0.01$, $r=0.71$), and CD4 T-cells ($z=-4.4$, $p<0.01$, $r=0.52$). In contrast, the Ad26.COV.2 vaccine induced a significantly higher CD8 T-cell response as compared to the mRNA vaccine ($z=-4.1$, $p<0.01$, $r=0.48$). Spike-specific IgG antibody levels and neutralizing activity as well as spike-specific CD4 and CD8 T-cell levels after vaccination are illustrated in figure 3.

Immune response after heterologous vaccination

A second heterologous mRNA vaccination with BNT162b2 was recommended for all individuals who had received a single dose of Ad26.COV.2. This led to a significant increase in both humoral and cellular immune responses (figure 3). IgG-levels increased from a

	mRNA			Ad26.COV.2		
Parameter	before	after	p-value	before	after	p-value
Spike specific IgG antibodies	4 BAU/ml (IQR 4 BAU/ml)	5702 BAU/ml (IQR 4343 BAU/ml)	<0.01	4 BAU/ml (IQR 2 BAU/ml)	61 BAU/ml (IQR 52 BAU/ml)	<0.01
Spike specific Neutralizing antibodies	0 % (IQR 0%)	99% (IQR 0.48%)	<0.01	0 % (IQR 0%)	11% (IQR 24%)´	<0.01
Spike specific CD4 T-cells	0 % (IQR 0.01%)	0.13 % (IQR 0.12%)	<0.01	0 % (IQR 0.01%)	0.05% (IQR 0.05%)	<0.01
Spike specific CD8 T-cells	0 % (IQR 0.005%)	0.02% (IQR 0.06%)	<0.01	0 % (IQR 0.003%)	0.15% (IQR 0.19%)	<0.01

Table 1 Blood parameters before and after vaccination with mRNA and Ad26.COV.2. Spike-specific IgG antibody levels [BAU/ml], neutralizing activity [%IC50], and the percentage of spike-specific CD4 and CD8 T-cells were quantified after two doses of a mRNA vaccine (n=56; BNT n=53, mRNA-1273 n=3) and after a single dose of Ad26.COV.2 (n=16), as well as before those vaccinations. Median values and interquartile range (IQR) are given.

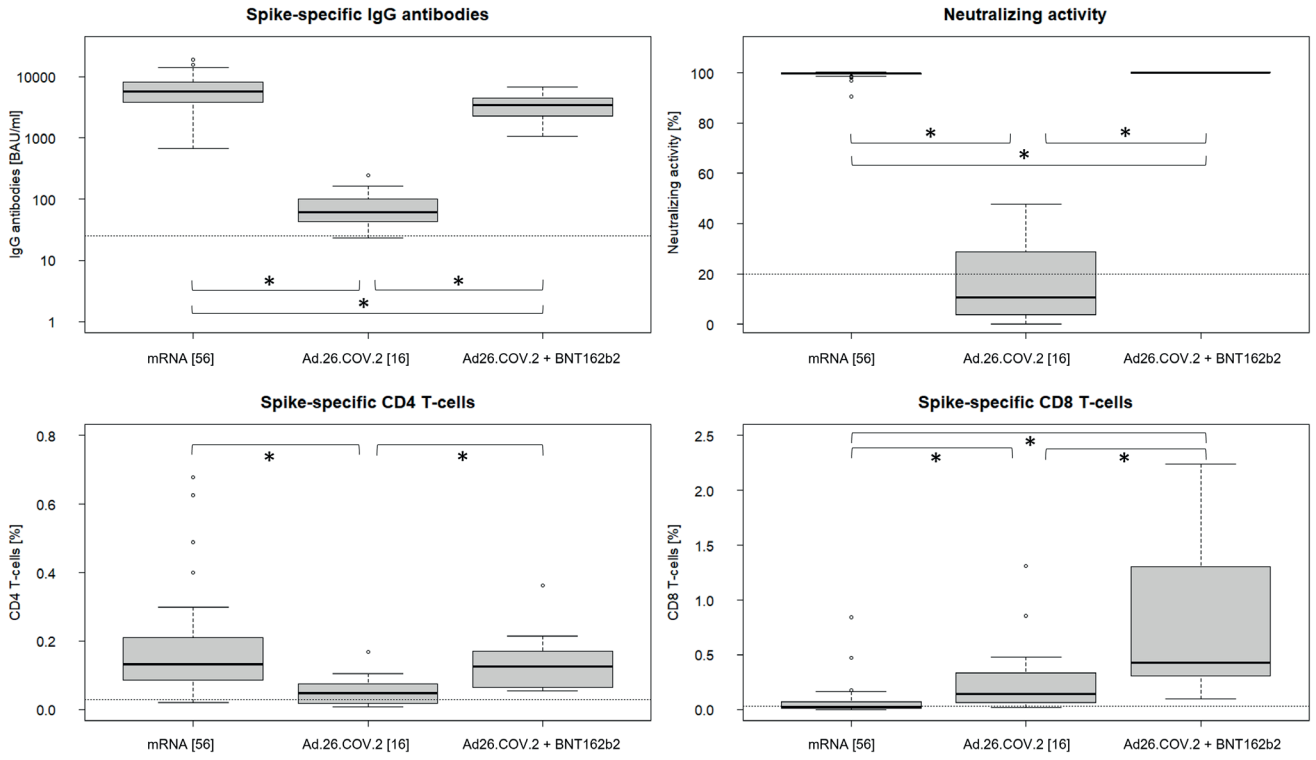

Figure 3. Vaccine-induced antibodies and T cells. Median spike-specific IgG antibody levels [BAU/ml], neutralizing activity [%IC50], and the percentage of spike-specific CD4 and CD8 T-cells were quantified after two doses of a mRNA vaccine (n=56; BNT n=53, mRNA-1273 n=3), a single dose of Ad26.COV.2 (n=16) or after heterologous combination of Ad26.COV.2 followed by BNT (n=11). Thresholds defining a negative response are indicated by a stippled line. Asterisks mark significance <0.05.

median of 61 BAU/ml (IQR 52 BAU/ml) to a median of 3456 BAU/ml (IQR 2209, z=-3.3, p<0.01, r=0.88) and the neutralizing activity from a median of 11% (IQR 24) to 100% (IQR 0.24, z=-3.3, p<0.01, r=0.88). Likewise, spike-specific CD4 T-cells increased from a median of 0.05% (IQR:0.05) to 0.13% (IQR 0.1, z=-2.6, p<0.01, r=0.75) and the CD8 T-cells from a median of 0.15% (IQR:0.19) to 0.43% (IQR 1, z=-2.6, p<0.01, r=0.75).

Comparison of the immune response after mRNA vaccine regimen and adjusted regimen

When compared to the homologous mRNA double dose vaccination regimen, IgG antibody levels after heterologous vaccination were moderately lower (z=-2.6, p<0.01, r=0.32), while the neutralizing activity (z=-3.6, p<0.01, r=0.45) and the CD8 T-cell response (z=-4.8, p<0.01, r=0.58) were significantly more pronounced. No difference was observed in CD4 T-cell levels (z=-0.6, p=0.54).

Long-term immune response after mRNA vaccine regimen

For the mRNA vaccines, all four chosen indicators significantly decreased after 6 months: IgG from a median of 5702 BAU/ml (IQR 4343 BAU/ml) to 1043 BAU/ml ((IQR 1112 BAU/ml), z=-7.7, p<0.01, r=0.87), neutralizing activity from a median of 99% (IQR 0.48) to 98% ((IQR 6), z=-4.8, p<0.01, r=0.70), CD4 T-cells from a median of 0.13 % (IQR 0.12) to 0.03% ((IQR 0.03),z=-5.9, p<0.01, r=0.86) and CD8 T-cells from a median of 0.02% (IQR:0.06) to 0.01% ((IQR 0.02),z=-3, p<0.01, r=0.45).

Due to necessary adaptions of the study design and limited numbers, a long-term follow-up after a single dose-vector vaccine (marginal reaction after 3 weeks) or heterologous regimen after Ad.26.COV.2 prime (too much delay) was not performed.

Adverse vaccine reactions

After the first dose of the mRNA vaccine, all athletes reported pain at the injection site lasting for a median time of 3 days (IQR 1). The most frequently reported systemic side effect was fatigue with 70% (median time: 2 days, IQR 3 days) and headache with 45% (median time: 0 days, IQR 1 day). The second mRNA dose caused pain at the injection site in 76% of cases for a median time of 2 days (IQR 2 days). Fatigue was reported by 71% (median time: 2 days, IQR 3 days) and headache by 59% (median time: 1 days, IQR 3 days) of the athletes. After the Ad26.COV.2 dose, all athletes reported pain at the injection site for a median time of 4 days (IQR 2 days). Fatigue was reported by 93% (median time: 3 days, IQR 2 days) and headache by 87% (median time: 3 days, IQR 1 day) of the athletes. The second heterologous mRNA dose led to local pain in 92% (median time: 2 days, IQR 1 day). Fatigue was reported by 84% (median time: 3 days, IQR 3 days) and headache by 75% (median time: 2 days, IQR 2.5 days) of the athletes. Occurrence of all collected local and systemic side effects is shown in figure 4.

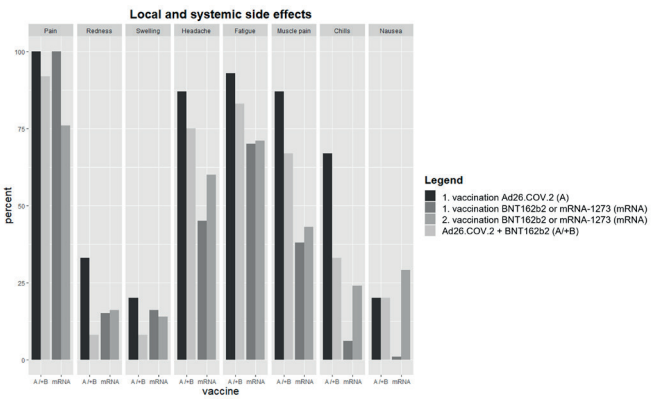

Figure 4. Local and systemic side effects. The different vaccine regimens are shown with their occurrence of local and systemic side effects.

Training Restrictions

Training restriction due to adverse events after the first and second mRNA vaccination lasted for a median of 2 days (IQR 1). The single dose Ad26.COV.2 vaccine led to a comparable training restriction of 2 (IQR1) days (z=-0.09, p=0.9, r=0.01). The heterologous regimen after Ad26.COV.2 priming was followed by a cumulative restriction of training of 3 days (IQR 1), which was not significantly different from the two dose mRNA vaccines (z=-0.73, p=0.46, r=0.1).

DISCUSSION

The aim of our study was to evaluate the humoral and cellular response in elite athletes after vaccination with different regimes against COVID-19. The main findings were (i) the humoral and cellular immune response in athletes was induced after double-dose mRNA and single-dose vector vaccines, (ii) the mRNA and vector vaccines differed in their immunogenicity, with Ad26.COV.2 as single-dose being less potent for increasing IgG antibodies, neutralizing activity and CD4 T-cells, but more potent in inducing the CD8 T-cell response, (iii) a heterologous mRNA vaccination after Ad26.COV.2 priming was able to bring the humoral and cellular immune response close to double-dose mRNA vaccinations in all parameters, and (iv) there were no differences in training restrictions between the vaccine regimens. All side effects were minor and did not lead to substantial training loss.

Lo Sasso et al. (2021)(18) state that an effective immune response can be inferred from the increase in IgG antibodies and their related neutralizing activity, as well as from induction of CD4 and CD8 T-cells. Particularly, the neutralizing antibody titers are considered important for the protection against acquisition of SARS-CoV-2 infection due to their ability to inhibit spike protein attachment to the ACE-2 receptor, and consequently inhibit entry of the coronavirus (18). Initial studies on the immunogenicity of a single dose of the Ad26.COV.2 vaccine among non-athlete healthy individuals reported adequate induction of neutralizing antibody titers against the wild type and the Alpha variant, and some studies even showed durable and sufficient responses against new variants of the coronavirus (19–21). In contrast, the current study showed that the single dose of the Ad26.COV.2 vaccine only induced poor neutralizing antibody activity in elite athletes, which may indicate insufficient protection against infection and transmission. Similar findings have been reported for immunocompetent individuals in general by Self et al. (2021) (21) who claim that the single-dose vector vaccine is the least immunogenic one of the available vaccines. On the other hand, it induced a comparably strong CD8 T-cell response, which in concert with a low neutralizing antibody function may still protect from severe courses of COVID-19 disease once infected. Thus, the Ad26.COV.2 vaccine may protect athletes from serious outcomes of the infection, but it is potentially less effective in protecting against an acquisition of the infection and transmitting it to other athletes; it should therefore not be considered an effective choice for elite athletes participating in major sport events who want to avoid SARS-CoV-2 infections.

The double-dose mRNA vaccines showed a clearly stronger induction of neutralizing antibody titers and CD4 T-helper cells compared to the single-dose vector vaccine. This aligns with findings from Tada et al. (2021)(23) who showed significantly lower neutralizing antibody titers against all variants after Ad26.COV.2 compared to BNT162b2 and mRNA-1273. Collectively, findings support the notion of an inadequate humoral immune response after a single-dose vector vaccine, thereby explaining the increased rate of breakthrough infections (24), thus necessitating a second immunization following Ad26.COV.2 vaccine to increase protection from virus acquisition. Moreover, it is likely that transmission between athletes cannot be effectively prevented by the Ad26.COV.2 vaccine to control the virus spread in settings typical for sport and major sports events.

However, CD4 and CD8 T cells also contribute to the effectiveness of vaccinations. Grifoni et al. (2020)(25) showed that individuals who had contact with the virus develop CD4 T-cells in 100% and CD8 T-cells in 70% of cases and inferred that this mobilisation of the adaptive immune system may assist in the prevention of severe courses of COVID-19. In our study, the double-dose mRNA vaccinations led to a larger induction of CD4 T-cells than the single dose vector vaccine, whereas the latter induced a moderately higher CD8 T-cell response. Therefore, prevention of severe courses can be assumed for both vaccine regimen.

Under consideration of these findings, athletes vaccinated with Ad26.COV.2 were offered an additional vaccination to improve their immune response. A study by Atmar et al. (26) showed that the humoral immune response can be significantly improved with a heterologous boost after Ad26.COV.2 priming, leading to similar immune responses as homologous mRNA booster vaccination. Our data confirm these findings by showing a large improvement in all investigated immunological parameters. Moreover, a comparison of vaccine-induced immune responses after homologous mRNA vaccination with heterologous vector/mRNA vaccination in immunocompetent non-athlete individuals using exactly the same analysis methods also revealed significantly higher CD8 T-cell levels after heterologous vaccination, which is in line with our findings in elite athletes (16, 27). Accordingly, in October 2021, the Standing Committee on Vaccination (STIKO) at the Robert Koch Institute, the relevant council for vaccination policies in Germany, recommended a heterologous mRNA boost vaccination to all persons who have received the Ad26.COV.2 vaccine to optimize immunity against SARS-CoV-2 (24).

Typical vaccine related adverse events may lead to training restrictions and are therefore important aspects to consider when vaccinating athletes, particularly during their preparation for major sport events like Olympic Games. In the current study, there were no significant differences in (cumulative) training restrictions between the double-dose homologous mRNA, the single dose-vector vaccine, and the heterologous vector-mRNA regimens. Median training restriction was 2-3 days. In our study only training restrictions were considered that were caused by side effects with a score larger than 1, although it has to be taken into account that there may be additional reasons for athletes not to train than only side effects, e.g. general caution after vaccination. Comparable results have been found in British Olympic athletes where side effects after mRNA vaccination lasted for 1-2 days (28). Thus, adverse events in elite athletes appear to be generally

mild and short-lived with limited impact on training. However, individual athletes may be affected considerably longer (up to 9 days; (28)) so that - if possible - vaccinations should be planned well in advance of the next competition. Of note, training restrictions after vaccination are considerably lower and more predictable compared to an infection with SARS-CoV-2 (29).

Lastly, there was an expected large decline in the immune response 6 months after the double-dose mRNA vaccines. Accordingly, a third vaccine dose with BNT16b2 or mRNA-1273 can be considered to boost the immune response and increase the protective effect(30), which was generally recommended at a later stage of the pandemic.

Limitations

Due to the vaccine shortage and local differences in vaccine availability at the time of prioritizing Olympic Games aspirants for vaccination, it was not possible to control and randomize assignment of the vaccine regimens, which precluded a more rigorous study design. This is similar to many COVID-19 related studies, which arose from the circumstances at that time. Moreover, the time interval of the heterologous boost after the first dose of the Ad26.COV.2 vaccine was longer than between the first and second mRNA vaccinations, which may contribute to altered immune responses as compared to the dual dose mRNA regimen (31). However, at the time of planning the study, the less pronounced immune response after single-dose vector vaccine was unforeseeable. Altogether, some unpredictable changes in the national COVID-19 policy had a relevant influence on our study protocol without invalidating the measurements per se (but weakening the conclusions).

Perspective

This study helps to understand the induced immune response after COVID-19 vaccinations in athletes, and vaccine related training restrictions and side effects. In addition, it would be interesting to investigate the association of the analysed immune response with the number of athletes that experience SARS-CoV-2 infection, as well as the severity and duration of their symptoms. This could provide better insights in the actual risk of infections after vaccination and the protection that is assumed by the immune response. Another new question that can be explored in the future is more detailed analysis of the side effects. Detection of side effects and training limitations was performed in our study using paper-based questionnaires. It would also be interesting to investigate limitations using objective measurement devices including fitness watches or other biometric devices. These can detect parameters such as heart rate, heart rate variability, sleep phases and skin temperature that may be associated with the vaccination and documented side effects. This has been previously investigated using a wrist-worn biometric device, but not specifically in elite athletes(32).

Conclusion

In contrast to double-dose mRNA vaccination, a single-dose vector vaccination does not seem to protect athletes sufficiently against acquisition of COVID-19. Receiving a booster dose seems to induce a sufficient immune response in all cases. There were no indications for a compromised immune response to vaccination in elite athletes. Based on both the strong immunogenicity and limited side effets, this study does not provide any evidence against vaccinating elite athletes against COVID-19.

Acknowledgment

The authors thank Jan Wüstenfeld from Leipzig for his support in enrolling participants. The authors also thank all participants for taking part in this study.

Conflict of Interest and Source of Funding

M.S. has received honoraria for lectures or participation in advisory boards for Takeda, MSD, Moderna, Biotest, Novartis or Qiagen. BCG hast received honoraria for lectures or participation in advisory boards from Sanofi, Seqirus, GSK and BionTech. All other authors declare no conflicts of interest. This study was financially supported by the German Federal Institute of Sport Sciences (Bundesinstitut für Sportwissenschaften; reference: 2521BI0106) and part of a larger study being registered in the German Clinical Trials register (DRKS00023717).

REFERENCES

1. Pfortmueller CA, Spinetti T, Urman RD, Luedi MM, Schefold JC. COVID-19-associated acute respiratory distress syndrome (CARDS): Current knowledge on pathophysiology and ICU treatment – A narrative review. Best Pract Res Clin Anaesthesiol 35: 351–368, 2021. doi: 10.1016/j.bpa.2020.12.011.
2. Berlit P, et al. Neurologische Manifestiationen bei COVID-19 [Online]. www.awmf.org [27 Jul. 2022].
3. Lechien JR, Chiesa-Estomba CM, De Siati DR, Horoi M, Le Bon SD et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. European Archives of Oto-Rhino-Laryngology 277: 2251–2261, 2020. doi: 10.1007/s00405-020-05965-1.
4. Szekely Y, Lichter Y, Taieb P, Banai A, Hochstadt A et al. Spectrum of Cardiac Manifestations in COVID-19: A Systematic Echocardiographic Study. Circulation 142: 342–353, 2020. doi: 10.1161/CIRCULATIONAHA.120.047971.
5. Robert Koch-Institut (RKI). Leitfaden für den ÖGD zum Vorgehen bei Häufungen von COVID-19 [Online]. https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Leitfaden_OEGD_COVID-19.html [25 Jul. 2022].
6. Meyer T. Infection Managment in Elite Athletes. Aspetar Sports Medicine Journal 4, 2015.
7. Meyer T, Wolfarth B, Gärtner B. Recommendations for athletes to vaccinate against SARS-CoV-2. Deutsche Zeitschrift für Sportmedizin/German Journal of Sports Medicine 72: E1–E4, 2021. doi: 10.5960/dzsm.2021.486.
8. Chaudhary N, Weissman D, Whitehead KA. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov 20 Nature Research: 817–838, 2021.
9. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. New England Journal of Medicine 383: 2603–2615, 2020. doi: 10.1056/nejmoa2034577.
10. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. New England Journal of Medicine 384: 403–416, 2021. doi: 10.1056/nejmoa2035389.

11. Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. New England Journal of Medicine 384: 2187–2201, 2021. doi: 10.1056/nejmoa2101544.
12. Ständige Impfkommission am Robert-Koch-Institut. STIKO-Empfehlung zur COVID-19 Impfung [Online]. https://www.rki.de/DE/Content/Kommissionen/STIKO/Empfehlungen/Vierte_Empfehlung_2021-04-01.html [1 Nov. 2022].
13. Kakanis MW, Peake J, Brenu EW, Simmonds M, Gray B et al. The open window of susceptibility to infection after acute exercise in healthy young male elite athletes. Exerc Immunol Rev 16, 2010. doi: 10.1016/j.jsams.2010.10.642.
14. Tafuri S, Sinesi D, Gallone MS. Vaccinations among athletes: evidence and recommendations. Expert Rev Vaccines 16 Taylor and Francis Ltd: 867–869, 2017.
15. Schmidt T, Klemis V, Schub D, Schneitler S, Reichert MC et al. Cellular immunity predominates over humoral immunity after homologous and heterologous mRNA and vector-based COVID-19 vaccine regimens in solid organ transplant recipients. American Journal of Transplantation 21: 3990–4002, 2021. doi: 10.1111/ajt.16818.
16. Schmidt T, Klemis V, Schub D, Mihm J, Hielscher F et al. Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination. Nat Med 27: 1530–1535, 2021. doi: 10.1038/s41591-021-01464-w.
17. Schub D, Klemis V, Schneitler S, Mihm J, Lepper PM et al. High levels of SARS-CoV-2–specific T cells with restricted functionality in severe courses of COVID-19. JCI Insight 5, 2020. doi: 10.1172/jci.insight.142167.
18. Lo Sasso B, Giglio RV, Vidali M, Scazzone C, Bivona G et al. Evaluation of Anti-SARS-Cov-2 S-RBD IgG Antibodies after COVID-19 mRNA BNT162b2 Vaccine. Diagnostics 11: 1–9, 2021. doi: 10.3390/diagnostics11071135.
19. Jongeneelen M, Kaszas K, Veldman D, Huizingh J, Vlugt R Van Der. Ad26 . COV2 . S elicited neutralizing activity against Delta and other SARS - CoV - 2 variants of concern. bioRxiv x, 2021. doi: https://doi.org/10.1101/2021.07.01.450707.
20. Alter G, Yu J, Liu J, Chandrashekar A, Borducchi EN et al. Immunogenicity of Ad26.COV2.S vaccine against SARS-CoV-2 variants in humans. Nature 596: 268–272, 2021. doi: 10.1038/s41586-021-03681-2.
21. Barouch DH, Stephenson KE, Sadoff J, Yu J, Chang A et al. Durable Humoral and Cellular Immune Responses 8 Months after Ad26.COV2.S Vaccination. New England Journal of Medicine 385: 951–953, 2021. doi: 10.1056/nejmc2108829.
22. Self WH, Tenforde MW, Rhoads JP, Gaglani M, Ginde AA et al. Comparative Effectiveness of Moderna, Pfizer-BioNTech, and Janssen (Johnson & Johnson) Vaccines in Preventing COVID-19 Hospitalizations Among Adults Without Immunocompromising Conditions. MMWR Morb Mortal Wkly Rep 70: 1337–1343, 2021. doi: 10.15585/mmwr.mm7038e1.
23. Tada T, Zhou H, Samanovic MI, Dcosta BM, Cornelius A et al. Neutralization of SARS-CoV-2 Variants by mRNA and Adenoviral Vector Vaccine-Elicited Antibodies. Front Immunol 13, 2022. doi: 10.3389/fimmu.2022.797589.
24. Robert-Koch-Institut. 43 Epidemiologisches Bulletin [Online]. https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2021/Ausgaben/43_21.html [5 Oct. 2022].
25. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell 181: 1489-1501.e15, 2020. doi: 10.1016/j.cell.2020.05.015.
26. Atmar RL, Lyke KE, Deming ME, Jackson LA, Branche AR et al. Heterologous SARS-CoV-2 Booster Vaccinations - Preliminary Report. medRxiv x, 2021. doi: 10.1056/nejmoa2116414.
27. Klemis V, Schmidt T, Schub D, Mihm J, Marx S et al. Comparative immunogenicity and reactogenicity of heterologous ChAdOx1-nCoV-19-priming and BNT162b2 or mRNA-1273-boosting with homologous COVID-19 vaccine regimens. Nat Commun 13, 2022. doi: 10.1038/s41467-022-32321-0.
28. Hull JH, Wootten M, Ranson C. Tolerability and impact of SARS-CoV-2 vaccination in elite athletes. Lancet Respir Med 10: e5–e6, 2022. doi: 10.1016/S2213-2600(21)00548-8.
29. Krzywański J, Mikulski T, Krysztofiak H, Pokrywka A, Sobierajski T et al. Vaccine versus infection – COVID-19-related loss of training time in elite athletes. J Sci Med Sport 25, 2022. doi: 10.1016/j.jsams.2022.10.004.
30. Robert-Koch-Institut. 48 Epidemiologisches Bulletin [Online]. https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2020/Ausgaben/48_20.pdf?__blob=publicationFile [3 Oct. 2022].
31. Voysey M, Costa Clemens SA, Madhi SA, Weckx LY, Folegatti PM et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. The Lancet 397: 881–891, 2021. doi: 10.1016/S0140-6736(21)00432-3.
32. Presby DM, Capodilupo ER. Biometrics from a wearable device reveal temporary effects of COVID-19 vaccines on cardiovascular, respiratory, and sleep physiology. J Appl Physiol 132: 448–458, 2022. doi: 10.1152/japplphysiol.00420.2021.