Virus Research 364 (2026) 199691

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Replication-incompetent viral vaccine vectors ChAdOx1 and MVA as tools for evaluating T-cell responses to naturally processed antigens *in vitro*

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ARTICLE INFO

Keywords:
ChAdOx1
ChAdOx2
MVA
B cells
T-cell activation
Naturally processed antigens
Biosafety

ABSTRACT

Assessing T-cell responses is critical for vaccine development. In vitro methods using SARS-CoV-2 or recombinant vaccinia virus with B cells effectively activate T-cells but require stringent biosafety conditions. As an alternative, we explored attenuated, replication-incompetent viral vectors, such as modified vaccinia Ankara (MVA) and chimpanzee adenoviral vectors (ChAdOx1 and ChAdOx2). These vectors successfully transduced B cells, as confirmed by GFP expression. B cells transduced with ChAdOx1 nCoV-19 (encoding SARS-CoV-2 Spike) activated autologous CD8⁺ and CD4⁺ T-cells. Similarly, B cells transduced with MVA encoding Spike activated autologous CD4⁺ T-cells. Our findings provide proof-of-concept support for the use of these safer viral vectors in vitro studies of vaccine-induced cellular immunity.

1. Introduction

Evaluating T-cell responses to pathogen infection or vaccination is critical for vaccine development, offering insights into both humoral and cellular immunity. While traditional in vitro peptide pool assays have limitations in reflecting natural antigen processing (Van Tilbeurgh et al., 2021), a novel autologous B cell-based in vitro assay, utilising SARS-CoV-2 or recombinant vaccinia virus (rVACV), has been developed to enable proper natural antigen presentation (Yin et al., 2023). However, this approach presents significant biosafety challenges, particularly when handling infectious agents like SARS-CoV-2 (Wang et al., 2020). Also, the use of rVACV in laboratory settings presents significant biosafety challenges, primarily due to its potential to cause infection in humans, particularly in immunocompromised individuals, elderly, and pregnant women, and its ability to spread through aerosol transmission (MacNeil et al., 2009). To mitigate these risks, we investigated attenuated, replication-incompetent viral vectors, specifically modified vaccinia Ankara (MVA) and chimpanzee adenoviral vectors (ChAdOx1 and ChAdOx2) (Folegatti et al., 2022; Folegatti et al., 2019; Gilbert, 2013; Voysey et al., 2021). We observed that all modified viral vectors efficiently transduced Epstein-Barr virus (EBV) immortalised B cells from a SARS-CoV-2 convalescent patient. Furthermore, transduction of EBV-immortalised B cells with ChAdOx1 encoding SARS-CoV-2 Spike activated autologous CD8⁺ and CD4⁺ T-cells. Similarly, transduction of immortalised B cells with MVA encoding Spike elicited autologous CD4⁺ T-cell activation. These findings demonstrate that replication-deficient viral vaccine vectors serve as valuable tools for investigating immune mechanisms in vitro in settings requiring stringent biosafety.

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https://doi.org/10.1016/j.virusres.2026.199691
Received 6 November 2025; Received in revised form 2 January 2026; Accepted 9 January 2026
Available online 10 January 2026
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2. Material and methods

2.1. Cells and reagents

All culture media, material and chemicals described here were purchased from Thermo Fisher Scientific (Waltham, MA, USA), unless indicated otherwise. EBV-immortalised B cells derived from SARS-CoV-2 convalescent patients or ChAdOx1 nCoV-19 immunised individuals were a kind gift of Prof. Tao Dong from the Chinese Academy of Medical Sciences Oxford Institute (CAMS/COI), and their immortalisation procedure was described previously (Dong et al., 2004; Yin et al., 2023). Autologous CD8^{+} and CD4^{+} *T*-cells from one of the convalescent patients (1493) were also provided by Prof. Tao Dong and their characterisation is detailed elsewhere (Yin et al., 2023). Ethical committee approval was obtained from a National Health Service (NHS) Research Ethics Committee (REC Number: 20/SC/0179). All necessary UK permissions were obtained via the Integrated Research Application System (IRAS Reference: 281,904), ensuring compliance with ethical and regulatory standards for clinical research.

2.2. Recombinant viral vectors

All viral vectors used in this work were supplied by the Viral Vector Core Facility (VVCF) from the Pandemic Sciences Institute (PSI) of the University of Oxford (Oxford, UK). The MVA vaccine vector was originally developed from VACV in Germany in the 1970s through more than 570 serial passages of the latter viral strain in primary chick embryo fibroblast (CEF) cells (Hochstein-Mintzel et al., 1972; Mayr et al., 1978). The vector was later transferred to the Jenner Institute at the University of Oxford (Schneider et al., 1998), and subsequent genetic engineering optimised it to enhance immunogenicity and improve manufacturing yield (Alharbi, 2019; Bošnjak et al., 2021; Ewer et al., 2013; Rampling et al., 2016). ChAdOx1 and ChAdOx2 are derived from chimpanzee adenoviral parental strains Y25 and C68 (Pan 9), respectively, which were originally isolated more than 50 years ago (Basnight et al., 1971; Hillis and Goodman, 1969). The Y25 isolate was later provided to researchers at the Jenner Institute by Johns Hopkins University (Dicks et al., 2012), whereas the C68 (Pan 9) isolate was subsequently obtained by the Jenner Institute, University of Oxford, from the American Type Culture Collection (ATCC) (Dicks et al., 2012). Subsequent work at the Jenner Institute led to the genetic engineering and optimisation of these viruses (Dicks et al., 2012; Joe et al., 2022; van Doremalen et al., 2020), resulting in the replication-deficient ChAdOx1 and ChAdOx2 adenoviral vaccine vectors used today.

Sequences used in this work were SARS-CoV-2 Spike (Genbank YP_009724390.1) and GFP (Genbank ANC98519.1).

2.3. EBV-immortalised B cells transduction

The EBV-immortalised 1493 B cells mentioned above were transduced with the viral vectors MVA nCoV-19 GFP, ChAdOx1 GFP, ChAdOx2 GFP, and ChAdOx1 nCoV-19, as previously described (Kim et al., 2004; Yin et al., 2023). Additionally, ChAdOx1 GFP was used to evaluate the transduction levels of several EBV-immortalised B cells derived from volunteers vaccinated with a single dose of ChAdOx1 nCoV-19 or from SARS-CoV-2 convalescent patients. As for EBV-immortalised 1493 B cells, these immortalised B cells were also produced as previously described (Yin et al., 2023). Briefly, the viral vectors were diluted in R0 medium (RPMI without supplements), were added to the B cells at the specified multiplicity of infection (MOI), i.e., number of virus used per each cell. The cells were incubated for 90 min at 37 °C in 5 % CO_2 to facilitate viral entry. After this incubation period, R10 medium was added to the cells and they were incubated for the duration specified according to each experiment.

2.4. Transduction efficiency assessment

The transduction efficiency of each viral vaccine vector in EBV-immortalised 1493 B cells was evaluated by quantifying GFP expression 16 h post-infection. B cells transduced with MVA nCoV-19 GFP, ChAdOx1 GFP, and ChAdOx2 GFP at different MOI were centrifuged and washed with PBS (Merck Life Sciences, Darmstadt, Germany). Subsequently, a 1:200 dilution of the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit was added to the wells containing the B cells, and the samples were incubated at 4 °C for 15 min in the absence of light. Following incubation, the cells were centrifuged, the supernatant was discarded, and the cells were fixed at 4 °C for 30 min using the Intracellular Fixation and Permeabilisation Buffer Set from eBioscience™ (San Diego, CA, USA). After fixation, FACS buffer was added, the cells were centrifuged, and the supernatant was removed. The cells were then resuspended in FACS buffer for data acquisition. All experiments were performed in triplicate, with each sample from each well transferred to individual FACS tubes. Data were acquired using the Aurora spectral cytometer (Cytek Biosciences, Fremont, CA, USA) equipped with Spectral Flow software (Cytek Biosciences). Gating strategy was optimised following previously defined protocols (Bošnjak et al., 2021; Douradinha et al., 2011; Joe et al., 2022; Palma et al., 2017; Palma et al., 2019; van Doremalen et al., 2020). The transduction efficiency of ChAdOx1 GFP was also evaluated both in EBV-immortalised 1493 B cells at several MOI and time points post-infection and in EBV-immortalised B cells derived from other SARS-CoV-2 convalescent patients or from volunteers who had received a single dose of ChAdOx1 nCoV-19. In these experiments, to establish the gating strategy, APC/Cyanine7 anti-human CD19 antibody (BioLegend, San Diego, CA, USA) was added to one set of B cell samples, while its corresponding isotype control, mouse IgG1 kappa isotype control (P3.6.2.8.1), was added to a separate set of B cell samples, following the manufacturer's instructions.

2.5. In vitro evaluation of T-cell responses induced by autologous EBV-immortalised 1493 B cells transduced with the viral vaccine vectors MVA nCoV-19 GFP and ChAdOx1 nCoV-19

EBV-immortalised 1493 B cell lines were transduced with the MVA nCoV-19 GFP and ChAdOx1 nCoV-19 as described above. After incubation, the cells were counted and co-cultured with autologous 1493 CD8^{+} or CD4^{+} T-cells at an effector-to-target (E:T) ratio of 1:1 for 4 h, following previously reported protocols (Peng et al., 2020; Reiné et al., 2011; Yin et al., 2023). Negative controls included 1493 B cells transduced with MVA GFP or ChAdOx1 GFP. To exclude dead cells, samples were stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit as explained previously. Fixation and permeabilisation were performed overnight as above using the Intracellular Fixation and Permeabilisation Buffer Set (eBioscience™). Subsequently, CD8^{+} T-cells were stained with BV421-anti-CD8 (BioLegend), PE-anti-interferon-γ (IFN-γ; BD Biosciences), and APC-anti-tumour necrosis factor-α (TNF-α; eBioscience, 1:500). CD4^{+} T-cells were stained with BV421-anti-CD4 (Thermo Fisher), PE-anti-IFN-γ (BD Biosciences), and APC-anti-TNF-α (BioLegend). Positive controls were stimulated with both CD3 monoclonal antibody (UCHT1) and PMA/ionomycin (Reiné et al., 2011; Yin et al., 2023). Unless indicated otherwise, all antibodies were added to the cell pools at a dilution of 1:33. CD8^{+} and CD4^{+} *T*-cells were considered activated by the autologous 1493 B cells when expressing both IFN-γ and TNF-α (Reiné et al., 2011; Yin et al., 2023).

2.6. Data analysis

The data were analysed using FlowJo V10.10 (BD Biosciences, Franklin Lakes, NJ, USA) and Spectral Flow software 3.0.1 (Cytek Biosciences), using an unmixing matrix derived from cells and compensation beads (BD Biosciences).

3. Results

3.1. *Attenuated viral vectors MVA GFP, ChAdOx1 GFP and ChAdOx2 GFP effectively transduce EBV-immortalised B cells derived from a SARS-CoV-2 convalescent patient*

MVA, an attenuated poxvirus extensively studied as a vaccine vector, is known to transduce a variety of human and animal cell lines, as reviewed elsewhere (Verheust et al., 2012). In this study, we used an MVA vector encoding a reporter gene, the green fluorescent protein (GFP), MVA GFP. The transduction ability of this vector was evaluated in EBV-immortalised 1493 B cells derived from a SARS-CoV-2 convalescent patient (Yin et al., 2023). Using an optimised gating strategy (Fig. 1A), we observed that MVA GFP efficiently transduced 1493 B cells, even at a low MOI (Fig. 1B). This was evidenced by a clear population of GFP-expressing cells, confirming effective transduction.

ChAdOx1 and ChAdOx2 adenoviral vectors are also known for their broad ability to efficiently transduce a variety of cell types (Folegatti et al., 2022; P.M. 2019; Voysey et al., 2021). We subsequently assessed the ability of ChAdOx1 GFP and ChAdOx2 GFP to efficiently transduce 1493 B cells. While both adenoviral vectors successfully transduced 1493 B cells, as evidenced by GFP expression and using a gating strategy similar to that employed for MVA GFP, a higher MOI was required for these adenoviral vectors compared to MVA GFP (Fig. 1C). Furthermore, ChAdOx1 GFP exhibited higher levels of transduction at the same MOI, as indicated by its more robust GFP expression compared to ChAdOx2 GFP (Fig. 1C). We also conducted a time-course experiment using ChAdOx1 GFP to transduce 1493 B cells at varying MOI of 10, 50, and 100, with transduction assessed at multiple time points between 16 and 48 h. We first optimised a flow cytometry gating strategy (Fig. 2A). As expected, the highest transduction efficiency was observed at a MOI of 100. Transduction levels at this MOI exhibited minimal variation across the assessed time points (Fig. 2B). Therefore, for all subsequent experiments, a MOI of 100 was selected, and GFP expression was evaluated 16 h post-incubation.

We also assessed the ability of ChAdOx1 GFP to transduce additional EBV-immortalised B cells derived from other SARS-CoV-2 convalescent patients and from volunteers immunised with a single dose of the ChAdOx1 nCoV-19 vaccine. Similar to the 1493 B cells, these B cells were immortalised following the same protocol and have previously been utilised to establish a model for natural antigen processing by B cells and subsequent T-cell activation (Yin et al., 2023). ChAdOx1 GFP was observed to efficiently transduce all EBV-immortalised B cells, regardless of origin, although transduction levels varied among them (Supplementary Fig. 1).

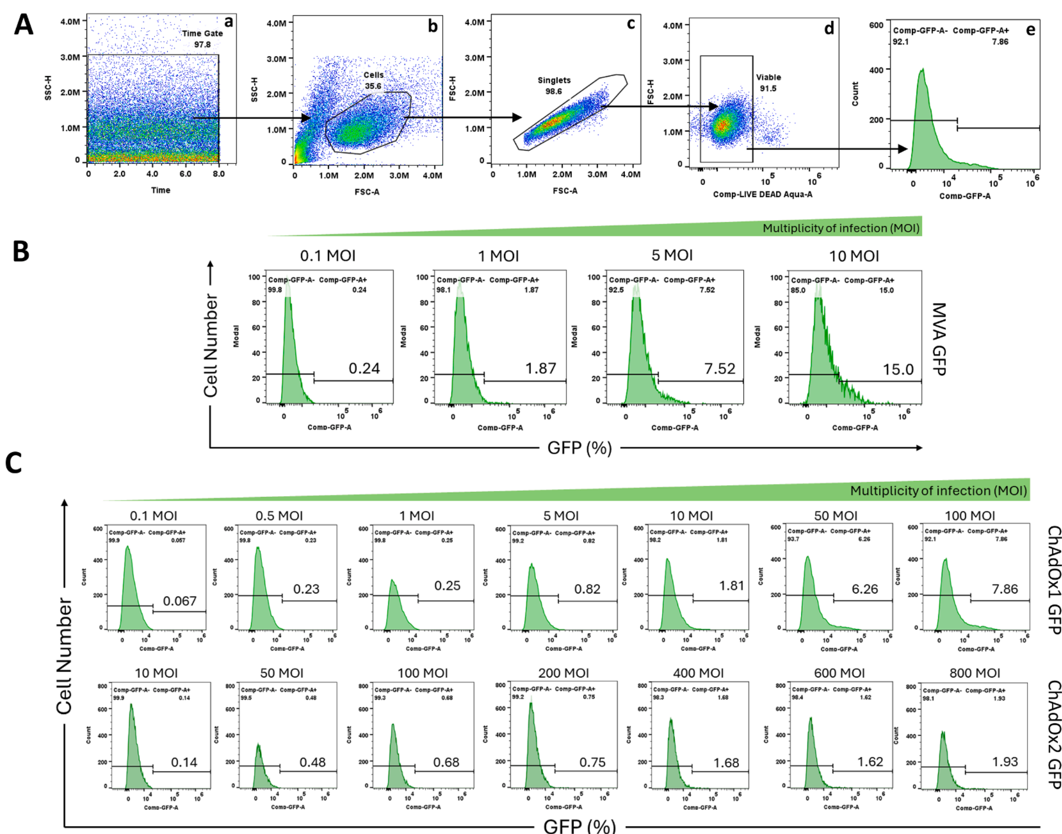

Fig. 1. Titration of MVA GFP, ChAdOx1 GFP and ChAdOx2 GFP in human EBV-transformed 1493 B cells derived from a SARS-CoV-2 convalescent patient. To assess the transduction efficiency of these attenuated viral vectors, various MOI were tested. The cells were transduced with MVA GFP at MOI of 0.1, 1, 5 and 10, with ChAdOx1 GFP at MOI of 0.1, 0.5, 1, 5, 10, 50, and 100, and with ChAdOx2 GFP at MOI of 10, 50, 100, 200, 400, 600, and 800. All titrations were performed approximately 16 h post-transduction. **A**, Proposed gating strategy for identifying transduced cells from a representative sample. a) time gating to determine acquisition periods without disruptions. b) Accurate identification of the EBV-transformed cell population based on their light scatter parameters (FSC and SSC-A). c) Identification of singlets to enhance result accuracy. d) Identification of viable cells. e) Histogram displaying the percentage of transduction, represented by the proportion of GFP-positive cells per sample. **B**, comparative histograms illustrating the percentage of viral transduction following transduction at different MOI for MVA GFP. Numbers above the histogram gate for GFP-positive cells indicate the percentage of positivity. **C**, comparative histograms showing the percentage of viral transduction following transduction at different MOI for ChAdOx1 GFP (upper panel) and ChAdOx2 GFP (lower panel). Numbers above the histogram gate for GFP-positive cells indicate the percentage of positivity.

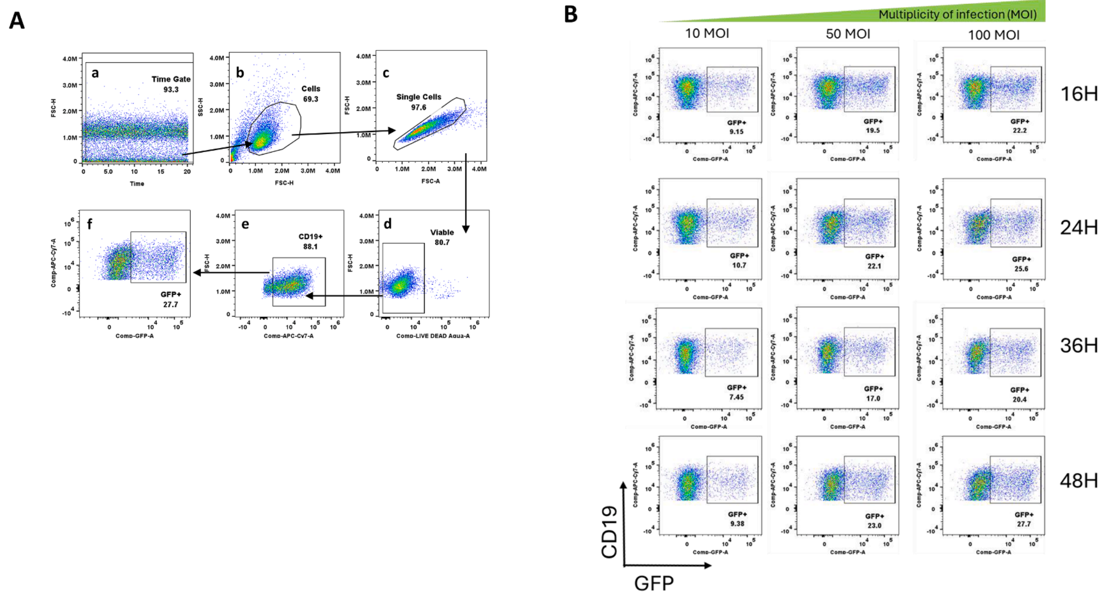

Fig. 2. Time-course analysis of ChAdOx1 GFP transduction efficiency in 1493 B cells at different MOI. A, gating strategy employed to identify and quantify 1493 B cells transduced by ChAdOx1 GFP at various time points. a) Time gating to isolate acquisition periods free from disruptions. b) Precise identification of the EBV-transformed cell population based on their forward and side scatter properties (FSC-A and SSC-A). c) Singlet gating to improve the accuracy of the results. d) Viable cell gating to exclude dead cells. e) Dot plot representative of B cells (CD19^{+}-cells). f) Dot plot representation of the B cell transduction efficiency, expressed as the percentage of GFP-positive cells within the sample. **B**, 1493 B cells were transduced with ChAdOx1 GFP at different MOI (10, 50, and 100). Transduction efficiency was assessed by measuring GFP expression at 16, 24, 36, and 48 h post-transduction.

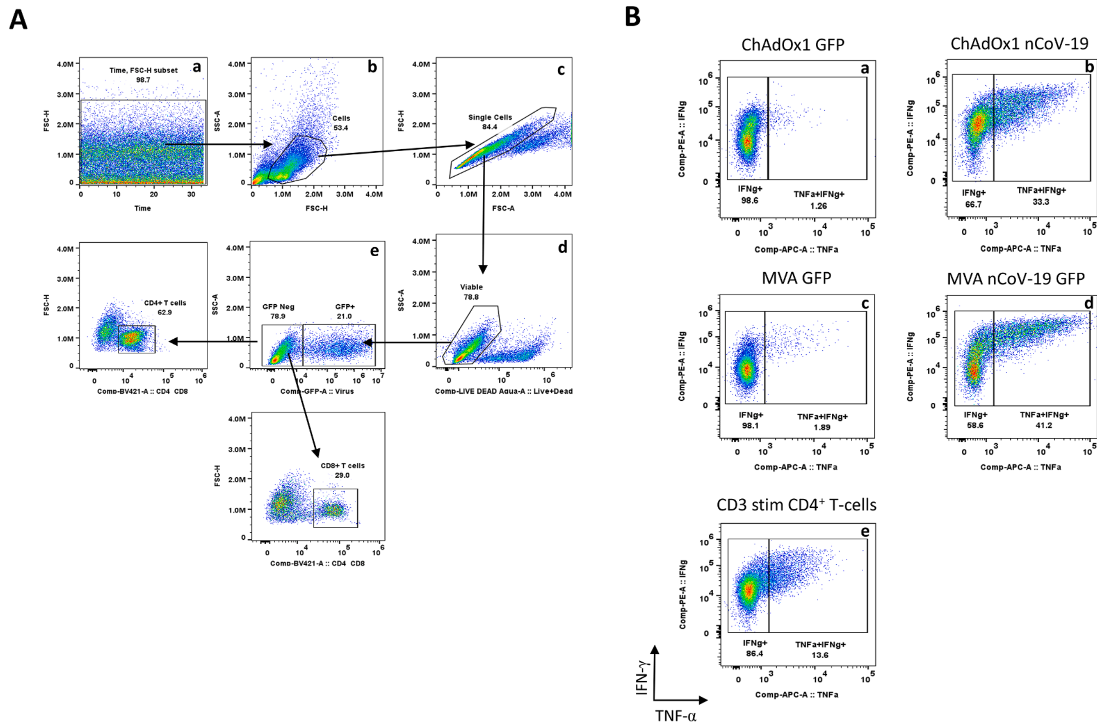

Fig. 3. Evaluation of SARS-CoV-2 Spike specific CD4^{+} *T*-cell response to autologous EBV-immortalised 1493 B cells transduced with either MVA nCoV-19 GFP or ChAdOx1 nCoV-19. To determine whether EBV-immortalised 1493 B cells transduced with either MVA nCoV-19 GFP or ChAdOx1 nCoV-19 could effectively stimulate autologous CD4^{+} *T*-cells, the B cells were initially transduced with MVA nCoV-19 GFP or ChAdOx1 nCoV-19 at a MOI of 10 and 100, and co-cultured with autologous CD4^{+} *T*-cells the following day. **A**, proposed gating strategy for identifying activated CD4^{+} *T*-cells from a representative sample: a) time gating to determine acquisition periods without disruptions; b) accurate identification of the CD4^{+} *T*-cell population based on their light scatter parameters (FSC and SSC-A); c) identification of singlets to enhance result accuracy; d) identification of viable cells, e) of GFP-negative cells and f) of CD4^{+} *T*-cells. Also, g) identification of CD8^{+} *T*-cells was performed using a similar gating strategy. **B**, Dot plots illustrating the percentage of activated CD4^{+} *T*-cells measured by IFN-γ and TNF-α release following co-culture with autologous B cells transduced with a) ChAdOx1 GFP, b) ChAdOx1 nCoV-19, c) MVA GFP, d) MVA nCoV-19 GFP, and e) stimulated with CD3 monoclonal antibody (UCHT1) and PMA/ionomycin (stim CD4^{+} *T*-cells). Numbers under the gate indicate the percentage of both cytokines' positive CD4^{+} *T*-cells.

3.2. EBV-immortalised 1493 B cells, transduced with either MVA nCoV-19 GFP or ChAdOx1 nCoV-19, elicited strong cellular responses in autologous T-cells in vitro

After confirming that the viral vaccine vectors MVA and ChAdOx1 efficiently transduce EBV-immortalised 1493 B cells, we evaluated whether these B cells, transduced with viral vectors encoding the SARS-CoV-2 Spike antigen sequence, could activate autologous T-cells. Although ChAdOx2 also efficiently transduced 1493 B cells, it required a higher MOI compared to ChAdOx1 (Fig. 1C). Consequently, subsequent T-cell activation experiments were conducted with the MVA and ChAdOx1 viral vectors. As for the previous experiments, we previously optimised a gating strategy which would allow us to quantify activated T-cells (Fig. 3A). We observed that 1493 B cells transduced with both MVA nCoV-19 GFP and ChAdOx1 nCoV-19 elicited a robust CD4⁺ T-cell response (Fig. 3B). Additionally, ChAdOx1 nCoV-19 also induced a strong CD8⁺ T-cell response (Fig. 4). These T-cell responses were specific to the SARS-CoV-2 Spike antigen and not attributable to the viral vectors themselves, as 1493 B cells transduced with MVA GFP or ChAdOx1 GFP failed to activate autologous CD4⁺ T-cells (Fig. 3B) and, in the case of the latter vector, CD8⁺ T-cells (Fig. 4).

Interestingly, although ChAdOx1 exhibited lower B cell transduction efficiency compared to MVA (Figs. 1B and 1C), the CD4⁺ T-cell responses induced by both viral vectors were robust (Fig. 3B), and did not reflect the observed differences in transduction efficiency, which confirms the suitability of ChAdOx1 for evaluating T-cell responses in vitro.

4. Discussion and conclusion

Many techniques assess cellular responses, but most do not account for natural antigen processing by APCs like B cells. While rVACV or live pathogen infection of patient-derived B cells can address this (Yin et al., 2023), such approaches require stringent biosafety conditions due to risks of transmission and infection, particularly with rVACV's potential for aerosol spread and risks to vulnerable populations (MacNeil et al., 2009; Wang et al., 2020). These constraints highlight the need for safer, accessible, and biologically relevant T-cell evaluation methodologies. Replication-incompetent viral vectors, including MVA, ChAdOx1, and ChAdOx2, offer a viable solution. These vectors have proven safe and immunogenic in numerous clinical trials for diseases like COVID-19, MERS, malaria, and HIV (Folegatti et al., 2022; P.M. 2019; Gilbert, 2013; Voysey et al., 2021). During the COVID-19 pandemic, adenoviral vector-based vaccines proved essential in reducing viral transmission and disease severity (Sampson et al., 2025; Voysey et al., 2021), similar to the impact of mRNA and DNA vaccines (Cagigi and Douradinha, 2023). These replication-incompetent vectors offer a safer alternative for T-cell evaluation by circumventing biosafety challenges of live pathogens and rVACV.

Given their favourable safety profiles, we evaluated MVA, ChAdOx1, and ChAdOx2 for their ability to transduce B cells as safer alternatives for in vitro immunological evaluations. Among the three viral vectors tested, MVA exhibited the highest capacity to transduce EBV-immortalised 1493 B cells. Since MVA, like rVACV, is derived from

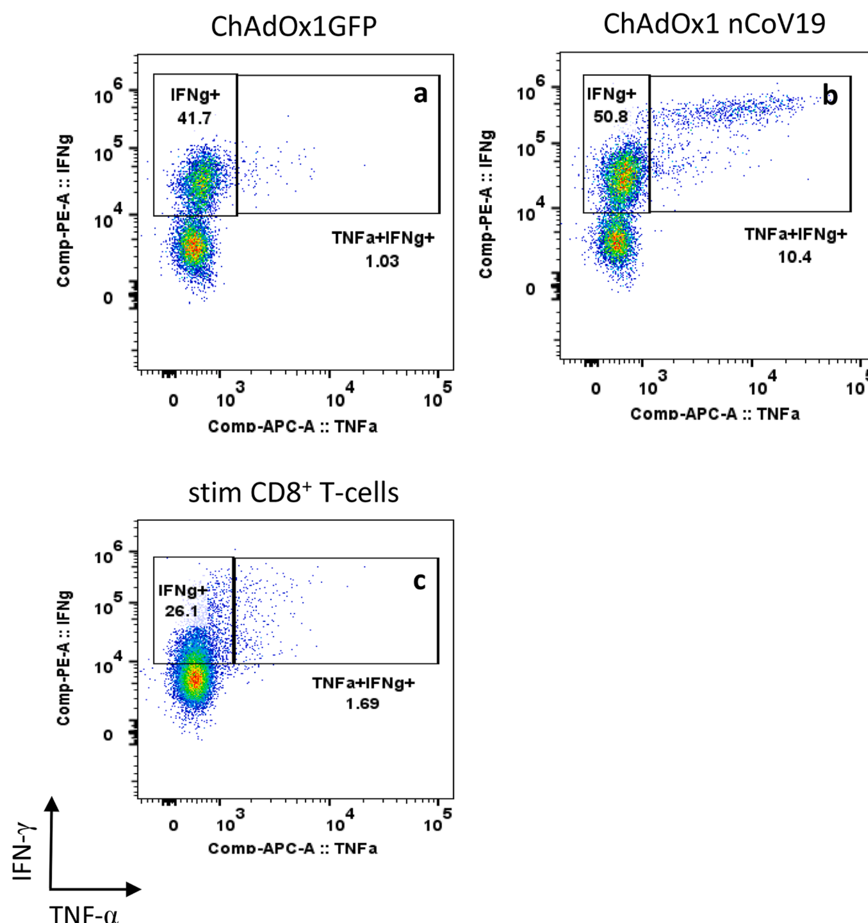

Fig. 4. Evaluation of SARS-CoV-2 Spike specific CD8⁺ *T*-cell response to autologous EBV-immortalised 1493 B cells transduced with ChAdOx1 nCoV-19. The ability of EBV-immortalised 1493 B cells transduced with ChAdOx1 nCoV-19 to activate autologous CD8⁺ *T*-cells was assessed using the same protocol and gating strategy as described in Fig. 3A. Dot plots display the percentage of activated CD8⁺ *T*-cells, also quantified by IFN-γ and TNF-α release after co-culture with autologous 1493 B cells transduced with a) ChAdOx1 GFP, b) ChAdOx1 nCoV-19, and c) stimulated with CD3 monoclonal antibody (UCHT1) and PMA/ionomycin (stim CD8⁺ *T*-cells). Numbers under the gate indicate the percentage of both cytokines' positive CD8⁺ *T*-cells.

the vaccinia virus, and rVACV has been shown previously to efficiently transduce EBV-immortalised B cells derived from both SARS-CoV-2 convalescent patients and volunteers vaccinated with a single dose of ChAdOx1 nCoV-19 (Yin et al., 2023), no issues were anticipated regarding the transduction of 1493 B cells by MVA. Similarly, ChAdOx1 and ChAdOx2 also transduced 1493 B cells, although at lower levels compared to MVA. Among the adenoviral vectors, ChAdOx2 appeared less effective at transducing EBV-immortalised 1493 B cells than ChAdOx1. Interestingly, this trend was also observed in the human RAMOS B cell line (Nazki, submitted). Moreover, ChAdOx2 has consistently been shown to induce both robust humoral and T-cell responses in animal models and human volunteers, confirming its immunogenic potential (P. M. Folegatti et al., 2019; Jenkin et al., 2022). However, for evaluation of T-cell responses in vitro, ChAdOx1 is a better option, due to its higher transduction level of 1493 B cells. The suitability of ChAdOx1 was further validated by assessing its transduction levels at different MOI and time points post-transduction in 1493 B cells. Additionally, we observed that ChAdOx1 also transduces EBV-immortalised B cells derived from other SARS-CoV-2 convalescent patients and from clinical trial volunteers who had received a single dose of ChAdOx1 nCoV-19.

Consistent with previous observations using SARS-CoV-2 or rVACV (Yin et al., 2023), transduction of B cells with MVA nCoV-19 or ChAdOx1 nCoV-19 led to high levels of autologous $CD4^+$ T-cell activation. ChAdOx1 nCoV-19 also activated autologous $CD8^+$ T-cells. These robust responses, comparable to those elicited by rVACV (Yin et al., 2023), confirm MVA and ChAdOx1 as viable and safer alternatives for in vitro T-cell evaluation. Furthermore, while previous studies using SARS-CoV-2-infected B cells (genetically modified for ACE-2 overexpression) showed higher T-cell activation (Yin et al., 2023), our vectors offer a safer alternative, avoiding the need for such modifications or replication suppression measures required with pathogenic strains.

Given the limited number of donors and the restricted availability of primary autologous T-cell subsets, this study should be considered a proof-of-concept demonstrating the feasibility of using replication-incompetent viral vaccine vectors to evaluate naturally processed, antigen-specific T-cell responses in vitro. Other limitations to this approach include variability in ChAdOx1 transduction efficiency across immortalised B cells derived from both convalescent patients and clinical trial volunteers, and challenges with limited primary cell availability, particularly for specific T-cell subsets. For instance, restricted availability of 1493 $CD8^+$ T-cells led to choosing ChAdOx1 over MVA for some experiments. We ultimately selected ChAdOx1, since, as previously mentioned, both MVA and rVACV are derived from vaccinia virus. Given the success of rVACV in transducing B cells and subsequently activating autologous T-cells, it was anticipated that MVA would exhibit a comparable effect on T-cell activation. We anticipate optimisation protocols will ease cell number constraints. Furthermore, adenoviral transgene expression is known to be time-dependent and influenced by vector batch characteristics, including viral particle-to-infectious unit ratios (Nazki, submitted), which can result in transient fluctuations in reporter expression without affecting overall transduction efficiency (Chu et al., 2003; Li et al., 2010). In addition, B cells are known to be relatively less permissive to adenoviral entry due to lower expression of canonical adenovirus receptors (Kim et al., 2004; Luisoni and Greber, 2016; Rice-Boucher et al., 2023), which can further contribute to variability in transduction efficiency and reporter expression across experiments. However, a systematic comparison of transduction efficiency across B cells derived from different origins was beyond the scope of this proof-of-concept study and is addressed in a separate, dedicated manuscript (Nazki, submitted). We also anticipate that similar donor-to-donor variability would be observed using B cells from individuals vaccinated with other platforms (e.g., mRNA), although formal cross-platform comparisons were outside the scope of this proof-of-concept study. While we recognize that generating immortalized B cell lines requires expertise, we believe that, in pandemic settings, having access to a system that bypasses live pathogen culture and animal use is a highly ethical, scalable and effective alternative. Animal models and subsequent immunogenicity analyses with peptide pools, though valuable, do not always mirror human HLA-restricted responses (Van Tilbeurgh et al., 2021; Yin et al., 2023). Our system proposes a humanised, HLA-relevant platform that can be established once per donor and reused for multiple vaccine antigen comparisons.

In this study, we demonstrate that both ChAdOx1 and MVA can be effectively utilised to assess T-cell responses in vitro. We propose their use for experimental approaches that rely on alternatives requiring higher biosafety measures, such as rVACV or infectious viral strains. This approach is particularly significant in the context of a pandemic, where access to laboratories of biosafety level 3 (BSL-3) or higher level, especially in developing countries, may be severely limited, restricting experimental work with pandemic-prone pathogens (Cagigi et al., 2024; Farias et al., 2023). Moreover, although demonstrated here using SARS-CoV-2 Spike as a model antigen, this workflow is readily adaptable to other pathogen-derived antigens encoded within replication-incompetent viral vectors. These viral tools would be particularly relevant to obtain correlates of cellular immune responses for pathogens which epidemics occur sporadically yet can cause severe morbidity or mortality, such as Chikungunya and Zika viruses (Cagigi et al., 2024). ChAdOx1 and MVA platforms have the advantage of being rapidly and easily engineered to incorporate the genetic sequence of a target antigen. This capability enables their application in laboratories with lower biosafety requirements, in that way facilitating the investigation of T-cell responses to specific antigens. Furthermore, this methodology supports the evaluation of immune responses to emerging variants of concern, which may arise locally within the regions housing these laboratories. By overcoming the constraints imposed by limited biosafety infrastructure, these vectors represent a valuable tool for advancing our understanding of cellular immunity in safer and resource-limited conditions.

Funding

This study was funded by the Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Science (CIFMS), China (SN and SG; grant number: 2018-I2M-2-002).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 4.0 (OpenAI, San Francisco, CA, USA) in order to improve the readability of the article. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Data and materials availability

Requests for data, materials, and reagents should be directed to the corresponding author. For materials and reagents provided as gifts, requests will be referred to the original donor(s). Requests specifically concerning the viral strains utilised in this study should be addressed to the Viral Vector Core Facility at the Pandemic Sciences Institute, University of Oxford (vvcf-enquiries@ndm.ox.ac.uk). Please note that these requests may be subject to a material transfer agreement (MTA).

CRediT authorship contribution statement

Salik Nazki: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Jesús Reiné:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Reshma Kailath:**

Validation, Resources, Methodology, Investigation, Data curation. **Sarah Gilbert:** Funding acquisition. **Bruno Douradinha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Salik Nazki and Sarah Gilbert reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by Chinese Academy of Medical Sciences. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Professor Tao Dong, Dr. Yanchun Peng, and Dr Ji-Li Chen, from CAMS/COI for generously providing the EBV-immortalised B cells derived from SARS-CoV-2 convalescent patients and ChAdOx1 nCoV-19-vaccinated volunteers, respective autologous T-cells, and for sharing their expertise in the immunological assays detailed in this work. We also extend our thanks to the Viral Vector Core Facility (VVCF) at the Pandemic Sciences Institute (PSI), University of Oxford, for their production of the viral vectors used in this research. We also thank to the Editor and the reviewers for their insightful and helpful comments and suggestions, which helped us to improve the scientific quality of this manuscript and rendered it more scientifically accurate.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2026.199691.

Data availability

Data will be made available on request.

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