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Research Paper

Heterologous Two-Dose Vaccination with Simian Adenovirus and Poxvirus Vectors Elicits Long-Lasting Cellular Immunity to Influenza Virus A in Healthy Adults

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ABSTRACT

Background: T-cell responses against highly conserved influenza antigens have been previously associated with protection. However, these immune responses are poorly maintained following recovery from influenza infection and are not boosted by inactivated influenza vaccines. We have previously demonstrated the safety and immunogenicity of two viral vectored vaccines, modified vaccinia virus Ankara (MVA) and the chimpanzee adenovirus ChAdOx1 expressing conserved influenza virus antigens, nucleoprotein (NP) and matrix protein-1 (M1). We now report on the safety and long-term immunogenicity of multiple combination regimes of these vaccines in young and older adults.

Methods: We conducted a Phase I open-label, randomized, multi-center study in 49 subjects aged 18–46 years and 24 subjects aged 50 years or over. Following vaccination, adverse events were recorded and the kinetics of the T cell response determined at multiple time points for up to 18 months.

Findings: Both vaccines were well tolerated. A two dose heterologous vaccination regimen significantly increased the magnitude of pre-existing T-cell responses to NP and M1 after both doses in young and older adults. The fold-increase and peak immune responses after a single MVA-NP + M1 vaccination was significantly higher compared to ChAdOx1 NP + M1. In a mixed regression model, T-cell responses over 18 months were significantly higher following the two dose vaccination regimen of MVA/ChAdOx1 NP + M1.

Interpretation: A two dose heterologous vaccination regimen of MVA/ChAdOx1 NP + M1 was safe and immunogenic in young and older adults, offering a promising vaccination strategy for inducing long-term broadly cross-reactive protection against influenza A.

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1. Introduction

Influenza A virus (IAV) remains a significant global health problem causing seasonal epidemics and occasional pandemics. Vaccination is the most cost-effective public health intervention to combat influenza (Petrie et al., 2015). Current seasonal influenza vaccines induce humoral immune responses to external glycoproteins, hemagglutinin (HA) and neuraminidase (NA). However, the error-prone nature of influenza virus replication leads to the accumulation of drift mutations within antigenic sites, allowing escape from serological immunity conferred by prior infection or vaccination. The requirement to make advance predictions of which viruses to include in vaccines for the forthcoming influenza season can result in vaccine mismatches (Pebody et al., 2015). Additionally, responses to seasonal influenza vaccines are subtype-specific, only inducing immune responses to strains included in the vaccine and offer no heterosubtypic protection against novel subtype reassortants or emerging viruses like H5N1 or H7N9 avian influenza. This is particularly critical in the elderly in whom vaccine efficacy is lower, increasing their risk for severe illness (Jefferson et al., 2005; Haq and McElhaney, 2014; Rivetti et al., 2006).

A protective role for $CD4^+$ and $CD8^+$ T-cells in humans has been demonstrated in experimental challenge studies (McMichael et al., 1983b; Wilkinson et al., 2012). More recently, in community cohort

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studies, T-cells have been shown to be associated with reduced viral shedding and limited severity of illness. Sridhar et al. identified a correlation between the frequency of IFN-γ^+/IL-2^- $CD8^+$ T-cells and protection against symptomatic influenza (Sridhar et al., 2013). Hayward and colleagues found that higher frequencies of nucleoprotein (NP)-specific IFN-γ^+ $CD3^+$ T-cells were associated with a lower risk of symptomatic, PCR-confirmed influenza infection and viral shedding (Hayward et al., 2015). As the induction of such cross-protective T-cells following vaccination with current influenza vaccines is limited (He et al., 2006), alternative vaccination approaches to induce T-cell responses against highly conserved internal influenza antigens capable of protecting against antigenically distinct viruses with pandemic potential, such as NP or matrix protein 1 (M1), are needed. This would particularly benefit high-risk populations, such as the elderly, in whom there is a high risk of severe disease.

We have developed viral vectored vaccines, using the replication-deficient chimpanzee adenovirus ChAdOx1 and the attenuated orthopoxvirus modified vaccinia virus Ankara (MVA) expressing NP and M1 influenza virus antigens (ChAdOx1 NP + M1 and MVA-NP + M1) as one approach to combat this problem. We have previously demonstrated that a single dose of these viral vector vaccines is safe and immunogenic (Antrobus et al., 2012; Lillie et al., 2012; Antrobus et al., 2014b) and in a proof-of-concept experimental influenza challenge study, showed that vaccination with MVA-NP + M1 can reduce the duration of viral shedding (Lillie et al., 2012). However, whether a vaccination strategy using a heterologous combination of these two viral vectors is synergistic in inducing higher magnitude, improved quality and longer durability of T-cell responses, as seen with other antigens, is not known. We conducted this randomized, open-label, Phase I clinical trial to assess the safety and cellular immunogenicity of prime/boost vaccination regimes employing MVA-NP + M1 and ChAdOx1 NP + M1 in young and older adults.

2. Materials and Methods

2.1. ChAdOx1 NP + M1 and MVA-NP + M1 Vaccines

Both vaccines have been described previously and consist of viral vectors expressing NP and M1 antigens from influenza A virus (H3N2, A/Panama/2007/99) as a single fusion protein (Antrobus et al., 2014b; Berthoud et al., 2011). MVA-NP + M1 was administered at a dose of $1{\cdot}5 \times 10^8$ plaque forming units (pfu) in 1·15 ml while ChAdOx1 NP + M1 was administered at a dose of $2{\cdot}5 \times 10^{10}$ viral particles (vp) in 0·22 ml.

2.2. Study Design and Participants

The study was a Phase I open-label, randomized, multi-center study conducted at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, UK, Surrey Clinical Research Centre, University of Surrey, UK and NIHR Wellcome Trust Clinical Research Facility, Southampton, UK (Table 1) (CONSORT diagram: Figs. 1 and 2). Healthy adults aged 18–46 (Groups 1–4) and 50 years or over (Groups 5 and 6) (Table 1) were eligible to participate in the trial after providing written informed consent. Full details of eligibility criteria are described in the trial protocol provided in the Supplementary material. All volunteers were healthy adults with negative pre-vaccination tests for HIV antibodies, hepatitis B surface antigen, hepatitis C antibodies and urine pregnancy test. Written informed consent was obtained in all cases and the study was conducted in accordance with the principles of the Declaration of Helsinki. For Groups 1–4, participants were randomized in variable block sizes according to vaccine allocation (ChAdOx1 NP + M1 or MVA-NP + M1 as the first vaccine) but not according to interval duration, which was determined by the preference of the volunteer until groups were full. The same randomization method was used to randomize participants to group 5 (ChAdOx1 NP + M1 only), or 6 (ChAdOx1 NP + M1 followed by MVA-NP + M1 8 weeks later). This was an open label study with subjects and investigators unblinded to the allocated group but study personnel conducting the immunology assays were blinded to group allocation. The clinical trial was approved within the UK by the regulatory authority (reference 21,584/0311/001-0001) and the Oxfordshire National Research Ethics Service Committee (OXREC A 13/SC/0004). The trial is registered at www.clinicaltrials.gov (Identifier: NCT01818362).

2.3. Study Procedures

All volunteers in Groups 1–4 were vaccinated on the day of enrolment and either 8 or 52 weeks later (CONSORT diagram: Figs. 1 and 2). Volunteers in Group 5 were vaccinated with a single dose of ChAdOx1 NP + M1 on the day of enrolment and volunteers in Group 6 were vaccinated with ChAdOx1 NP + M1 on the day of enrolment followed 8 weeks later by MVA-NP + M1. All vaccines were administered by an intramuscular (*im*) injection into the deltoid region of the arm. Volunteers were reviewed in clinic 24 h after vaccination for potential adverse events (AE) and were provided with a diary card to record solicited and unsolicited AEs which was reviewed at follow-up visits. Blood samples for safety and immunogenicity were collected at each follow-up visit (see Table 1 for timings). Safety events were assessed as the occurrence of local and systemic reactogenicity signs and symptoms for 7 days following vaccination procedures. Occurrences of serious adverse events were assessed during the whole study duration and changes from baseline were used for safety laboratory measures. Interferon gamma Enzyme-Linked ImmunoSpot assays (ELISpots) were used as a marker of cell mediated response at baseline and different time points throughout the trial.

2.4. Statistical Analysis

As a Phase I study with no predefined hypotheses, formal power calculations were not performed. With 12 subjects per group (Group 1–4), it was estimated that there would be 88% power (alpha = 0·05) to observe a three-fold increase in T-cell response to NP and M1 pre-vaccination to peak levels post-vaccination, although this was not the primary endpoint. With 10 per group, we would have 80% power and 68% power with 8 per group. This informal power calculation was carried out based on immune responses obtained from our previous trials with single use of ChAdOx1 NP + M1 and MVA-NP + M1.

All participants were included in safety analysis with safety data presented according to frequency, severity and duration of adverse events. The primary immunogenicity analysis compared the area under the curve (AUC) of the T-cell response (IFN-γ SFC/million peripheral blood mononuclear cells [PBMCs]) from baseline to week 78 for Groups 1 + 2 vs 3 + 4, or from baseline to week 26 for Group 5 vs 6. For the primary analysis, a *t*-test was performed on the intention to treat (ITT) population. The AUC was calculated using the trapezium method. Where a response at a time point was missing, we took the mean of imputed values from twenty imputed datasets (generated using multiple imputation by chained equations and the predictive mean matching method). Secondary analyses were performed using a t-test on the available data only, to compare between pre-specific groups. No formal adjustment for multiple significance testing was carried out for this phase I study, but note that all other analyses were exploratory. Primary, secondary and post-hoc immunogenicity analyses were carried out using STATA 14.2: StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP.

Exploratory immunogenicity data were analyzed using GraphPad Prism version 5.04 for Windows (GraphPad Software Inc., California, USA) and non-parametric analyses. To compare ELISpot responses between selected, matched time-points in a group, a Wilcoxon matched-pairs signed rank test was used. To compare baseline IFN-γ ELISpot responses between G1-6 a 1-way ANOVA was used with Kruskal-Wallis

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