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Induction of CD8$^+$ T cell responses and protective efficacy following microneedle-mediated delivery of a live adenovirus-vectored malaria vaccine

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ABSTRACT

There is an urgent need for improvements in vaccine delivery technologies. This is particularly pertinent for vaccination programmes within regions of limited resources, such as those required for adequate provision for disposal of used needles. Microneedles are micron-sized structures that penetrate the *stratum corneum* of the skin, creating temporary conduits for the needle-free delivery of drugs or vaccines. Here, we aimed to investigate immunity induced by the recombinant simian adenovirus-vectored vaccine ChAd63.ME-TRAP; currently undergoing clinical assessment as a candidate malaria vaccine, when delivered percutaneously by silicon microneedle arrays. In mice, we demonstrate that microneedle-mediated delivery of ChAd63.ME-TRAP induced similar numbers of transgene-specific CD8$^+$ T cells compared to intradermal (ID) administration with needle-and-syringe, following a single immunisation and after a ChAd63/MVA heterologous prime-boost schedule. When mice immunised with ChAd63/MVA were challenged with live *Plasmodium berghei* sporozoites, microneedle-mediated ChAd63.ME-TRAP priming demonstrated equivalent protective efficacy as did ID immunisation. Furthermore, responses following ChAd63/MVA immunisation correlated with a specific design parameter of the array used ('total array volume'). The level of transgene expression at the immunisation site and skin-draining lymph node (dLN) was also linked to total array volume. These findings have implications for defining silicon microneedle array design for use with live, vectored vaccines.

1. Introduction

The case for the development of needle-free methods for delivering vaccines is particularly convincing within the context of diseases that are prevalent in resource-poor settings, such as malaria [1]. Despite six decades of research effort and with one candidate vaccine in late stages of evaluation, no licensed malaria vaccine yet exists [2]. A promising platform for eliciting cellular immunogenicity against malaria antigens is their delivery by recombinant viral vectors such as live, replication-deficient human and simian adenoviruses and modified vaccinia virus Ankara (MVA) [3,4]. Heterologous prime-boost combinations, where two different vectors encode the same malaria antigen, induce high levels of antigen-specific T cells in mice, non-human primates and in phase I and II clinical trials in naïve and malaria-experienced humans [5–9].

Chimpanzee adenovirus serotype 63 (ChAd63) has been administered to humans *via* ID and intramuscular (IM) injection [7,10].

Abbreviations: ID, intradermal; IM, intramuscular; ChAd63, chimpanzee adenovirus serotype 63; MVA, modified vaccinia virus Ankara; ME-TRAP, multiple epitope string fused to the *Plasmodium falciparum* thrombospondin related adhesion protein; PbCSP, *Plasmodium berghei* circumsporozoite protein; dLN, (skin-)draining lymph node; PBS, phosphate buffered saline; VP, viral particles; PFU, plaque forming units; PBMC, peripheral blood mononuclear cells.

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Whilst the ID route has demonstrated dose-sparing effects over other needle-based routes for a number of licensed vaccines, this route is technically difficult and has been associated with adverse events in delivery of other live vaccines [11,12]. Additionally, training of vaccinators and sharps disposal are costly logistical requirements of immunisation campaigns. A simpler, potentially safer method of delivering vaccines as compared to needle injection is percutaneous delivery facilitated by silicon microneedle arrays. Upon application to the skin, these micron-scale structures penetrate through the skin's outermost barrier layer, the *stratum corneum*. The transient microchannels created by silicon microneedles extend through the *stratum corneum* into epidermis and dermis provide conduits for the diffusion of topically applied vaccine into these underlying sites rich in antigen-presenting cells [13]. By virtue of their sharp tips and smoothness, these silicon microneedles enter tissue smoothly at low insertion forces without fracturing, delivering into both epidermal and dermal compartments as confirmed using an *ex vivo* porcine skin model and MVA expressing a fluorescent protein. In contrast, ID injection was found to deliver dye only into the dermis [13].

Silicon microneedle arrays have previously been used to facilitate delivery of MVA encoding the *Plasmodium berghei* circumsporozoite protein (PbCSP), inducing antigen-specific $CD8^+$ T cell responses in mice [13]. T cell responses induced by homologous prime boost with MVA.PbCSP were found to induce equivalent protection against live *P. berghei* sporozoite challenge as those induced by ID immunisation. However, homologous prime boost immunisation regimes (*i.e.* MVA/MVA) are weakly efficacious as compared to heterologous regimes such as simian adenovirus serotype 63 (ChAd63)/MVA. Consequently, whereas the MVA/MVA regimen presents a useful tool to assess differences in immunogenicity and protection in the mouse model, the ChAd63/MVA regimen investigated here has greater clinical applicability. We hypothesised that delivery of ChAd63.ME-TRAP to mice by silicon microneedles and MVA boosting would induce antigen-specific $CD8^+$ T cell responses. The antigen ME-TRAP, encoded by both ChAd63 and MVA, is a fusion protein consisting of a multiple epitope string containing the *P. berghei* PbCSP MHC Class I epitope Pb9 (SYIPSAEKI) fused to the *P. falciparum* thrombospondin-related-adhesive-protein (TRAP) [14]. Given that: (i) needle-and-syringe ID delivery of both prime and boost immunisations in this regimen generate high levels of protection against *P. berghei* challenge (80–100%, [5]) and, (ii) that protective efficacy of silicon microneedle delivery of MVA.PbCSP/MVA.PbCSP was found to be equivalent to ID delivery [13], we hypothesised that microneedle array delivery of ChAd63/MVA would induce equally high levels of protection as repeated ID-based immunisation. Others have found equivalent immunogenicity of adenoviral vaccines delivered by microneedle arrays as by needle-and-syringe [15,16], but this is the first study assessing the induction of T cell responses and protective efficacy of simian adenoviruses delivered by silicon microneedles. A second aim of this work was to investigate how specific design dimensions of microneedle arrays affect the resulting $CD8^+$ T cell response in ChAd63/MVA immunisation. Based on our previous findings [13], we hypothesised that the total array volume of a microneedle array would correlate with the primary and inversely correlate with the secondary $CD8^+$ T cell responses induced by ChAd63/MVA immunisation.

2. Materials and methods

2.1. Microneedle arrays

Silicon microneedle arrays were fabricated using a highly-precise, reproducible and quality-controlled wet etching process at an ISO9001-accredited facility at the Tyndall National Institute, Cork, as previously described [17]. The total microneedle array volume (hereafter termed 'array volume') is defined as the volume of a microneedle with a length, L and a base radius, r, by the total number of microneedles on the array, n, and is given by the formula: $(1/3\pi r^2 L)n$. Arrays were grouped into—'small', 'intermediate', 'large', and 'extra large' categories according to array volume (Table 1).

2.2. Mice and immunisations

Female BALB/c mice (6–8 weeks) were purchased from Harlan, UK. All experimental procedures were in accordance with the Animals (Scientific Procedures) Act 1986 and reviewed by the University of Oxford Animal Care and Ethical Review Committee. Mice were anaesthetised with inhaled isofluorane. Vaccines were diluted in endotoxin-free Phosphate Buffered Saline (PBS). Immunisations were performed as previously described [13]. Briefly, ID immunisations were administered into the dorsal ear pinna (25 μL per ear) using a 29G syringe. Alternatively, 5 μL of vaccine was placed onto the dorsal surface of each ear before applying an array mounted onto a hand applicator on top of the 5 μL vaccine drop using a force of approximately 10–20 N for 20 s [13].

2.3. Vaccines and schedules

ChAd63.ME-TRAP and MVA.ME-TRAP were generated as previously described [7,18]. ChAd63.ME-TRAP was delivered at a dose of 1×10^{10} viral particles (VP) and the MVA.ME-TRAP dose was 1×10^6 plaque forming units (PFU). Priming immunisations were given either by microneedle array or by ID injection as described above. Boost vaccinations of 1×10^6 PFU MVA.ME-TRAP were given either by microneedle array or ID 8 weeks following the prime, according to a pre-determined protocol [5]. Blood was taken for measurement of Pb9-specific $CD8^+$ T cell immunogenicity 3 weeks following the prime, or 2 weeks following the boost immunisation.

2.4. Assessment of immunogenicity

Cellular immunogenicity was determined using IFN-γ ELISPOT or by intracellular cytokine staining (ICS) after stimulation with peptide Pb9. These assays are described in detail in [18].

2.5. In vitro *luciferase assay*

To investigate transgene expression after ChAd63 delivery by microneedle arrays of different array volume or after ID delivery, we used a vector encoding *Photinus* luciferase (ChAd63.pLuc). Ears and dLN were harvested 24 h after ChAd63.pLuc delivery by a range of microneedle arrays or after ID injection. Ears were split down the coronal plane, cut into small pieces and incubated with 1 mg/mL collagenase IV (Gibco) and 0.02 mg/mL DNAase I (Sigma) for 37 °C for 30 min before inactivating with 200 μL foetal calf serum. Draining lymph nodes (dLN) were incubated with 3 mg/mL collagenase-dispase and 0.02 mg/mL DNAse I and incubated for 30 min at room temperature. Skin and dLN digests were passed through a 70 μm cell strainer and cells pelleted by centrifugation before re-suspending in PBS. Cells (1×10^6) were lysed using a BRIGHTGLO™ luciferase assay buffer, according to manufacturer's instructions (Promega). BRIGHTGLO™ beetle luciferin was added to lysed cell supernatant for 5 min. Transgene expression (luciferase activity, expressed in 'relative luciferase units' was measured in skin and dLN lysates using a Varioskan luminometer. Background activity of substrate in PBS was subtracted from test samples.

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