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Epidermal delivery of protein and DNA vaccines

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Targeting vaccines to the skin epidermis results in the activation of an immune inductive site that is rich in antigen-presenting cells. The superficial location of the skin makes it accessible to vaccine delivery. However, it is difficult to access the epidermis using needle and syringe delivery, and vaccine antigens are too large to be effectively delivered using standard topical formulations. Needle-free vaccine delivery systems have been developed for efficient delivery of particulate vaccines into the epidermal tissue. Particle-mediated epidermal delivery of DNA vaccines is based on the delivery of DNA-coated gold particles directly into the cytoplasm and nuclei of living cells of the epidermis, facilitating DNA delivery and gene expression. Alternatively, protein vaccines can be formulated into a dense powder, which can be propelled into the skin epidermis by epidermal powder immunisation using similar delivery devices and principles, but in this instance the protein is delivered to the extracellular space. Preclinical and clinical data will be reviewed, demonstrating applications of epidermal vaccine delivery to a wide range of experimental infectious disease vaccines.

Keywords: DNA vaccine, epidermis, gene gun, hepatitis B, immunisation, influenza, protein, vaccine

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1. The skin as a target for immunisation

Epidermal layers of the skin, gastrointestinal tract and mucosal surfaces form the first line of defence against pathogen access to the body. The outermost layer of the skin, the stratum corneum, acts as a mechanical barrier to entry of pathogens and macromolecules. In addition to the barrier function, the skin is a potent immune inductive site [1]. The underlying viable epidermis is laced with a network of antigen-presenting cells (APCs), the Langerhans cells (LCs), which act as sentinels to detect the presence of foreign materials. Unstimulated LCs express low levels of major histocompatibility complex (MHC) and costimulatory molecules [2]. The activation of LCs by antigen triggers a series of molecular events resulting in LC migration from the epidermis to the draining lymph nodes, where they initiate adaptive primary immune responses [3]. In the lymph node, activated LCs present antigen to both $CD4^+$ and $CD8^+$ T lymphocytes. Antigen presentation to T-cell subsets is dependent on several factors, including antigen source, route of antigen processing and cytokine production in the lymph node [4-6]. Antigen processing and presentation in the epidermis can be modulated locally by cytokines secreted by epidermal LCs and keratinocytes. Dendritic cells, including LCs, process both endogenous and exogenous antigens, including the capacity to process exogenous particulate and cell-associated antigen via the MHC class I pathway, using a cross-priming mechanism [6-8].

The majority of commercial vaccines are delivered to intramuscular or subcutaneous tissues by needle and syringe. These sites have far fewer resident dendritic cells than the epidermis. The epidermis also lacks vasculature and contains a low density of sensory nerve endings. Thus, epidermal immunisation can avoid the bleeding and pain that are sometimes associated with needle and syringe immunisation. These factors, combined

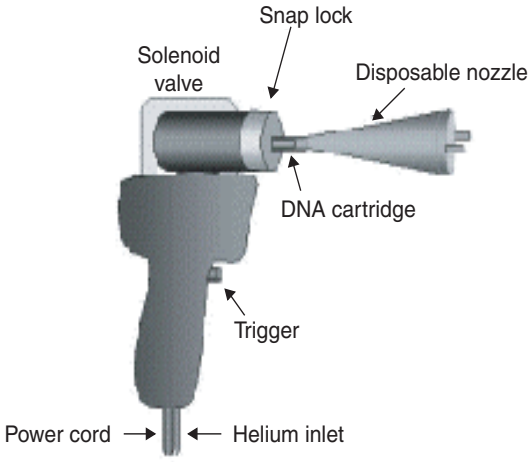

Figure 1A. Device for epidermal particle delivery. The prototype delivery device used for particle-mediated epidermal delivery of DNA vaccines is a gas discharge device. The system is a metal instrument that regulates a burst of helium with an electrical solenoid valve, which in turn powers delivery of particles to the skin. The DNA is contained within a cartridge inserted into a disposable nozzle, which is attached to the device with a snap lock. The device is actuated by pressing the trigger. This releases a small volume of compressed helium that exits the device into the bore of the DNA cartridge, stripping the DNA/gold from the tubing and accelerating the particles to high velocity. Particles then exit the nozzle in a circular pattern with enough force to penetrate the epidermal layer of the skin.

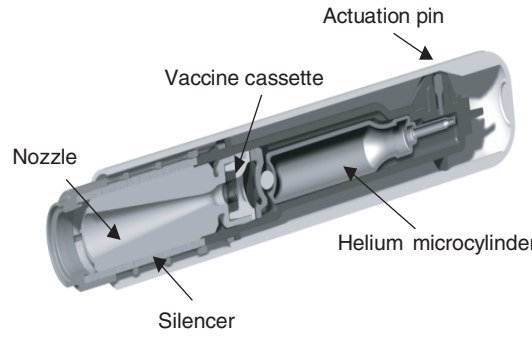

Figure 1B. Device for epidermal particle or powder delivery. Cross-sectional view of the single-use, disposable PowderJect ND5.2 device, used for epidermal powder immunisation of 20 – 70 mm protein powders. The device is composed of a gas bottle (5-cc volume), rupture chamber, a vaccine-containing cassette, a nozzle and a silencer. The gas bottle is filled with medical grade helium gas to a nominal pressure of 45 bar. The cassette (11-mm outer diameter, 6-mm internal diameter and 4-mm height) is constructed of a polyurethane thermoplastic elastomer washer with rupture membranes heat sealed to either side within which the powdered vaccine is housed. The rupture membranes are 20-mm thick semitransparent polycarbonate. On actuation of the device, the helium gas released from the gas bottle ruptures the membranes of the cassette and accelerates the vaccine powders into the viable epidermis. Each administration delivers 1 mg of powder.

with the abundance and superficial location of LCs, and their potent immune inductive activity, make the skin an attractive tissue for vaccine delivery. The challenge posed by targeting vaccines to the epidermis is to accurately and efficiently access the target tissue. Although the epidermis is superficially located and has a large surface area, access is restricted by the overlying stratum corneum, which forms a physical barrier to molecules > 500 Da molecular weight. In order to breach this barrier, high molecular weight antigens delivered topically must be given at a high dose and in combination with membrane-permeabilising agents such as cholera toxin [9]. Conversely, the epidermal layer is too thin to be reli-ably accessed by traditional needle and syringe injection. Targeted approaches are required to efficiently deliver vaccines to this potent immune inductive site. Several different epidermal vaccination strategies have been developed that employ particle or powder formulations of DNA and protein vaccines and devices to propel these particle-mediated or powdered vaccines specifically into the epidermis [10].

2. Powder and particle formulations and epidermal delivery systems

Powder- and particle-mediated vaccines are delivered to the skin using needle-free delivery systems to propel the particles into the viable epidermis. Most published studies have employed a multi-use research device (**Figure 1A**) for epidermal delivery of DNA vaccines, although one clinical study used a disposable, single-use device for epidermal delivery of a protein vaccine (**Figure 1B** [11]). These devices, developed by PowderJect Vaccines, Inc., employ helium as the motive force for the delivery of powdered or particulate vaccines into the skin. Once formulated into a powder or particles, the vaccine is loaded into a disposable cassette or cartridge and inserted into the device. Actuation of the device releases the pressurised helium, which liberates the vaccine particles, either by stripping them from the interior surface of the cartridge (**Figure 1A**), or by rupturing the membranes of the cassette (**Figure 1B**). The particles become entrained in the helium gas stream and gather momentum. Downstream of the cartridge or cassette, the device opens into a conical structure such that the helium gas expands outwards. This disperses the high-pressure jet of gas into a low velocity pulse, while the vaccine powder/particles maintain sufficient velocity to penetrate through the stratum corneum and deposit vaccine in the viable epidermis. Several device parameters can be fine-tuned to optimise particle delivery. Altering the helium pressure controls the depth of vaccine penetration into the skin, with optimal vaccine immunogenicity achieved by employing a pressure that delivers the vaccine into the epidermal layer. Lower pressures result in vaccine deposition on the skin surface and in the stratum corneum, leading to reduced delivery efficiency. Higher pressures propel a higher proportion of particles into the underlying dermis, which can lead to greater local reactogenicity.

Formulations have been developed with the physical properties, including particle size and morphology, density and dura-

Table 1. Efficacy of particle-mediated epidermal delivery of DNA vaccines in preclinical models.

Pathogen	Ref.
Viruses	
Influenza virus	[37,39,47,78-80]
Human immunodeficiency virus	[50,52,54]
Rotavirus	[81-83]
Canine and rabbit papillomaviruses	[33,84-86]
Rabies virus	[43,87]
Vaccinia and monkeypox viruses	[32,70,71]
Ebola and Marburg viruses	[31,44,72,73]
Foot and mouth disease virus	[88]
Tick-borne, Murray Valley and Japanese encephalitis viruses	[41,42,89,90]
Hantaviruses	[45,46,48,91,92]
Venezuelan equine encephalitis virus	[31]
Dengue virus	[49]
Bacteria	
Bacillus anthracis	[31,93,94]
Pseudomonas aeruginosa	[95,96]
Listeria monocytogenes	[40]
Borrelia burgdorferi	[97]
Mycobacteria	
Mycoplasma pulmonis	[98]
Parasites	
Plasmodium spp.	[74-77]

bility, necessary to penetrate the stratum corneum and deliver a payload to the viable epidermis. For particle-mediated epidermal delivery (PMED) of DNA vaccines, the formulation consists of plasmid DNA encoding the relevant antigens condensed onto 1 – 3 µm diameter gold particles [12]. Protein antigens can also be coated onto gold particles [10,13,14]. Dense powder formulations have been developed for epidermal delivery of protein antigens using an approach termed epidermal powder immunisation (EPI). Protein antigen is formulated with excipients such as trehalose, mannitol and dextran, and subjected to a spray-freeze-drying process to form dense particles of 20 – 70 µm in diameter [15]. Spray-freeze-drying combines atomisation to generate suitable size droplets with freezing followed by the removal of ice by sublimation under vacuum.

3. Particle-mediated epidermal delivery of DNA vaccines

The goal of PMED of DNA vaccines is to facilitate DNA delivery by propelling DNA-coated gold directly into the cytoplasm and nuclei of cells of the viable epidermis. Particle-mediated DNA delivery systems, or 'gene guns' have been used since the 1980s to directly transfect DNA into plant and mammalian cells in order to achieve transient or stable transgene expression. Successful genetic immunisation was first described in 1992, and a PMED delivery system was used in that study [19].

Following PMED delivery, cells of the viable epidermis, including professional APCs (LCs) and keratinocytes are transfected with the plasmid [3,20]. The majority of particles are delivered to the cytoplasm, whereas a fraction of the DNA-coated gold particles are delivered directly to the cell nucleus [21]. Successful intracellular plasmid delivery results in transient antigen expression in both keratinocytes and LCs. The expressed antigen is processed and presented via both the MHC class I and II pathways and induces both humoral and cellular immune responses. The ability to directly transfect the target cells results in high efficiency of PMED. Immune responses have be achieved in humans against PMED vaccines at plasmid doses of $\leq$ 10 µg [12,22-25] (Drape *et al.*, manuscript in preparation), whereas intramuscular administration of as much as 5-mg DNA i.m. has resulted in variable success [26-30]. The ability of PMED DNA vaccines to elicit immune responses with such small amounts of DNA could facilitate simultaneous delivery of multiple plasmids/genes to be simultaneously delivered for the induction of immunity to multiple antigens within a single vaccine [31,32].

4. Immunogenicity and efficacy of particle-mediated epidermal DNA vaccines in preclinical models

Immunogenicity and challenge efficacy of a wide range of PMED DNA vaccines directed at viral, bacterial and parasite antigens have been demonstrated in multiple animal models, including rodents, pigs, poultry and nonhuman primates (**Table 1**). In these preclinical models, PMED DNA immunisation has been shown to elicit immune responses, including neutralising antibody, mucosal antibody and cellular immunity. The parameters required to direct the immune responses have been the subject of considerable investigation [10,33-35]. In general, immune responses in rodents and nonhuman primates to PMED DNA vaccines can be elicited at DNA doses of $\leq$ 10 µg. When PMED DNA vaccines have been directly compared with intramuscular DNA delivery, epidermal delivery has been found to elicit stronger immune responses with much lower DNA doses [36-40].

PMED DNA vaccines can elicit high titre, long duration protective neutralising antibody responses in preclinical models. Antibody responses in three inbred strains of mice vaccinated with a Murray Valley encephalitis PMED vaccine were found to be long lived [41]. Challenge efficacy was demonstrated 1 year after vaccination of mice with a PMED DNA vaccine for tick-borne encephalitis virus [42]. Cynomologus monkeys given a single rabies PMED DNA vaccination were also protected from

challenge at 1 year [43]. Animals vaccinated with a PMED Ebola virus vaccine were partially protected from challenge at 9 months postvaccination [44]. Finally, antibody titres at protective levels were present 2 years following a PMED hantavirus vaccine ([45] (J Hooper, pers. comm.). In some cases, protective efficacy of PMED DNA vaccines has been correlated with neutralising antibody titres [42,45-47]. In other cases, there is evidence that the protective immune mechanism differs between conventional vaccines and PMED DNA vaccines [31,48,49]. Interestingly, the preclinical efficacy data for several PMED DNA vaccines are suggestive of sterilising immunity [42,45].

The ability of PMED DNA vaccines to elicit mucosal immune responses has also been demonstrated. A PMED vaccine against HIV was shown to elicit mucosal immune responses in rhesus macaques, and to protect against mucosal virus challenge [50]. This result confirms the ability of the skin to function as an inductive site for mucosal immune responses.

A significant benefit of PMED DNA vaccination is the ability to induce cellular immune responses and in particular $CD8^+$ cytotoxic T lymphocytes (CTL). Such immune responses are likely to be important for effective vaccines against chronic infectious diseases and cancer. From a mechanistic perspective, PMED DNA vaccination is tailored toward the induction of cellular immune responses to defined antigens, by direct transfection of LCs in the epidermis with the plasmid-encoding antigen(s), enabling *de novo* protein synthesis and subsequent endogenous antigen processing. Depending on the antigen, dosing regimen, target species and adjuvant used, both T helper type 1- (T_H1) and 2-type responses can be elicited by PMED [51]. Experimentally, PMED DNA vaccines have been shown to elicit cellular immune responses in several species, including mice, ferrets, pigs and monkeys [39,44,49,50,52,53]. In mice, a PMED DNA vaccine for Ebola elicited strong cytotoxic T-cell responses and protected mice from lethal challenge, despite low neutralising antibody titres [44]. A PMED DNA vaccine against Dengue virus elicited both cytotoxic T lymphocytes and neutralising antibodies in mice [49]. Protective effects of cellular responses was indirectly suggested, as monkeys vaccinated with the same vaccine were protected despite the lack of antibody responses. Studies of an HIV PMED DNA vaccine in rhesus macaques demonstrated the induction of T_H1-type responses [50]. Additional studies demonstrated that PMED DNA vaccination could prime T_H1 and CTL responses in DNA vaccine prime–recombinant viral vector boost immunisation regimens [54-57].

5. Clinical results with particle-mediated epidermal delivery DNA vaccines

The PMED clinical trials reported so far include several Phase I clinical trials to evaluate safety and immunogenicity of PMED vaccines against hepatitis B, malaria and influenza [12,22-25,58] (Drape *et al.*, manuscript in preparation). The PMED DNA vaccines have been used in different regimens, including a prime followed by several booster doses in naive subjects [12,25], or previously vaccinated nonresponders [24], as part of a heterologous prime–boost regimen with boosting via viral vector [22,23], and as a single dose in previously primed subjects [24] (Drape *et al.*, manuscript in preparation).

The initial PMED clinical trials demonstrated the ability of a DNA vaccine expressing hepatitis B surface antigen (HBsAg) to elicit humoral and cellular immune responses in healthy volunteers [12,25,58]. HBsAg naive subjects developed HBsAg-specific antibody responses and $CD4^+$ and $CD8^+$ IFN-γ enzyme-linked immunospot (ELISPOT) responses after a prime and one or two booster doses of 1, 2 or 4 μg DNA per dose. Immunogenicity of the HBV PMED DNA vaccine was also demonstrated in individuals who did not respond to conventional HBsAg vaccination [24]. A single PMED vaccination was sufficient to seroconvert four of five subjects who had previously failed to seroconvert to a standard three-dose regimen of the conventional HBsAg vaccine. In a second group of subjects who had remained unresponsive to six to nine doses of conventional HBsAg vaccine, two of six subjects seroconverted after one or two doses of the PMED HBsAg vaccine. In a third group of subjects, the PMED DNA vaccine was able to boost responses in five of five subjects who had previously been successfully vaccinated with the conventional HBsAg vaccine but whose antibody titres had waned.

A PMED malaria DNA vaccine has also been evaluated in the clinic, as part of a prime–boost regimen with a recombinant modified vaccinia virus Ankara (MVA) vector [22,23]. The antigen-encoding DNA used was the *Plasmodium falciparum* thrombospondin-related adhesion protein (*TRAP*) gene fused to a synthetic sequence of additional malaria B- and T-cell epitopes. In this study, PMED and intramuscular delivery of the DNA vaccine priming regimen were compared. Three-dose PMED and intramuscular DNA vaccination regimens both elicited T-cell ELISPOT responses, at DNA dose levels of 4 μg for PMED and 500 – 2000 μg for intramuscular vaccination. T-cell responses were increased following boost with the same malaria fusion gene delivered by modified vaccinia ankara (MVA) vector. PMED DNA vaccine priming resulted in T-cell responses after MVA boosting that were superior to those obtained by priming with intramuscular DNA vaccine. No severe or serious adverse effects of the DNA–MVA prime–boost regimen were detected [23]. Cellular immune responses were five to tenfold higher with the heterologous prime–boost regimen compared with either the MVA vector or DNA vaccine alone.

A Phase I study has recently been completed to assess safety and immune responses to a single PMED influenza DNA vaccination (Drape *et al.*, manuscript in preparation). The DNA vaccine encoded the haemagglutinin (HA) antigen gene from influenza strain A/Panama (H3N2). The HA antigen was selected based on preclinical studies demonstrating that DNA vaccines encoding HA of several different subtypes of influenza A and B viruses elicit neutralising antibody responses and protection against challenge in a number of species including

Table 2. Epidermal powder immunisation: antigens and adjuvants tested in preclinical models.

Agent	Ref.
Antigen	
Influenza (whole virion, split or subunit)	[14,15,62-64,66]
Influenza nucleoprotein peptide	[14]
Hepatitis B surface antigen	[13,14]
Diphtheria and tetanus toxoid	[65,67]
Equine herpesvirus	[99]
HIV gp120	[68]
Adjuvant	
Aluminium hydroxide and phosphate	[13,65,67]
QS-21	[63]
CpG	[13,65]
LT, CT, CTB, LTR72, LTK63	[62,63,66]

CT: Cholera toxin; CTB: Cholera toxin B subunit; LT: *Escherichia coli* heat-labile toxin; LTB: *Escherichia coli* heat-labile toxin B subunit.

mice, pigs, chickens, ferrets and horses [59,60]. In the clinical trial, three different DNA dose levels were evaluated: 1, 2 and 4 µg. Immunisation resulted in significant rises in the anti-influenza antibody geometric mean titres (GMT) at all dose levels, with higher and more consistent antibody titres occurring in subjects vaccinated with the higher dose levels.

PMED DNA vaccines have not been associated with systemic reactivity; however, mild-to-moderate local dermal reactions have been observed following epidermal delivery [12,24,25] (Drape *et al.*, manuscript in preparation). Regardless of the plasmid delivered, the typical local reaction was characterised by mild-to-moderate erythema, oedema and skin discoloration, and occasionally itch/discomfort, followed by mild superficial skin flaking. Infrequent local reactions have included petechiae, minor bruising and small scabs. Oedema was generally observed early in the postvaccination period, whereas erythema and skin discoloration persisted for a longer period of time. Whereas the early data provide an initial indication of the safety of PMED DNA vaccination, larger scale trials will be required to completely evaluate the safety profile of any specific DNA vaccine.

6. Delivery of protein vaccines by epidermal powder immunisation

Protein vaccines can also be delivered in powdered form to the viable epidermis. As is the case for PMED, EPI vaccination also results in the activation of and presentation by LCs resident in the viable epidermis [34]. Due to the larger size (20 – 70 µm) and high water solubility of the EPI particles, the antigen is deposited extracellularly, where the sugar formulation is rapidly dissolved by interstitial fluids. Antigen rapidly diffuses from the vaccination site, and is efficiently endocytosed by LCs. Initially, nearly all LCs in the vicinity of the target site can be shown to contain antigen [61]. Levels of antigen and antigen-containing LCs are high for 2 days following vaccination, and remain present at the site for ≤ 5 days. LCs containing antigen can be detected at 20-h postvaccination in the draining lymph nodes, where antigen presentation normally occurs and persist for several days [14,34]. The LCs directly contribute to the immune response. LCs isolated from EPI vaccination sites can be adoptively transferred to naive animals, resulting in strong antibody responses in the transfer recipient, and the depletion of LC at the immunisation site significantly reduced the immune responses to EPI vaccination [62]. EPI also increased the local production of TNF-α and IL-12, which may modulate or increase immune responses to the vaccine [13,63].

7. Immunogenicity and efficacy of epidermal powder immunisation in preclinical models

Numerous antigens, including whole virus, split, subunit, particulate and peptide compositions, have been delivered in powdered form and shown to be immunogenic in preclinical models (**Table 2**). The majority of the preclinical studies have focused on powdered influenza vaccine. In mice, EPI influenza vaccines elicit serum antibody titres that are significantly higher than those elicited with the same amount of antigen administered by the intramuscular or subcutaneous routes [64]. These responses are protective against a homologous mouse-adapted influenza A strain, and against an antigenically distinct strain within the same influenza type as the vaccine strain. The EPI influenza vaccine elicits primarily IgG1 antibodies on mice. In contrast to the mouse data, antibody titres were similar in rhesus macaques vaccinated for influenza by intramuscular and EPI routes [63].

Several adjuvants, including alum, CpG, QS-21 and bacterial toxins and derivatives, have been formulated with antigens into powders for EPI (**Table 2**). Serum antibody titres against influenza were elevated in mice when CpG oligonucleotide, cholera toxin (CT), cholera toxin B sub-unit (CTB), or *Escherichia coli* heat-labile toxin mutants (LTR72 or LTK63) were added to the vaccine formulation [13,14,62,63,65,66]. Antibody responses were also elevated in vaccinated monkeys after addition of the adjuvants QS-21, LTR72 or CT to the powder formulation [63]. Adjuvantation can effect the quality as well as the magnitude of the immune response. CT and CpG direct the immune response toward IgG1 or IgG2 antibodies, respectively [13,14,65]. As is the case with epidermal delivery of DNA vaccines, EPI with protein vaccines can result in the induction of mucosal antibody responses. Influenza-specific antibody responses were observed in mucosal secretions of mice vaccinated by EPI [14,63]. However, mucosal immune responses were low in mice vaccinated with formulations lacking an adjuvant. The adjuvants CpG, QS-21, LTR72 or CT markedly enhanced mucosal immune responses. IgA secreting cells were detected in tracheal and intestinal cultures from vaccinated mice, demonstrating the local origin of the mucosal antibodies.

Other antigens have been shown to be immunogenic when formulated into powders and delivered by EPI. These include peptide (influenza nucleoprotein [NP] peptide), particulate (HBsAg, equine herpesvirus), bacterial toxoid (diphtheria toxoid [DT]) and glycoprotein (HIV gp120) antigens (**Table 2**). In general, the results with other antigens have mirrored those observed for EPI flu vaccine. Powdered DT formulations delivered by EPI elicit higher antibody titres in mice compared with subcutaneous immunisation with the same antigen dose [65,67]. As was the case with EPI flu vaccine, EPI DT vaccine primarily stimulates the IgG1 antibody subclass when formulated with alum, and IgG2 when formulated with CpG. Similarly, antibody titres in mice vaccinated with an HIV gp120 EPI vaccine were 18- to 240-fold higher than in mice vaccinated intramuscularly [68]. Finally, HBsAg vaccine has been formulated into powders with alum or CpG adjuvants and delivered by EPI [13].

8. Initial clinical evaluation of epidermal powder immunisation influenza vaccine

The first clinical evaluation of a vaccine delivered by EPI was recently reported [11]. A powdered trivalent influenza vaccine was evaluated for safety and immunogenicity in a Phase I clinical trial. Groups of 12 subjects received either a single EPI vaccination (15 µg of each influenza strain), or two adjacent EPI (total of 30 µg of each influenza strain). A third group of subjects received the comparable liquid influenza vaccine (Fluvirin®; 15 µg of each influenza strain) by intramuscular injection. Systemic reactogenicity was similar between control and EPI vaccines, and site reactions following EPI were generally mild and self-limiting, resolving in a time frame roughly equivalent to the natural sloughing of the epidermis. Seroconversions, titre increases and GMT to all three influenza strains in the vaccine were equivalent or higher in EPI immunised groups than in controls. Both the magnitude of antibody responses and the percentage of subjects achieving an antibody titre predictive of protection (> 1:40) were comparable between needle injection and EPI immunisation when the 15-µg dose of vaccine was tested. Immune responses in subjects receiving EPI vaccine at the 30-µg dose level were significantly higher in comparison with the commercial vaccine control for two of the three virus strains contained in the vaccine.

9. Expert opinion

The defining characteristic of DNA vaccine delivery by PMED is the high efficiency of transfection of live cells in the skin epidermis, resulting in *de novo* antigen synthesis in the transfected cells. The unique mechanism of action of DNA vaccines leads to the induction of cellular immune responses and antibody responses, including high-titre neutralising antibodies and mucosal antibodies. Protective efficacy has been demonstrated in preclinical models for PMED DNA vaccines encoding antigens from viruses, bacteria, mycobacteria and parasites (**Table 1**), where the protective mechanisms include both neutralising antibody and cellular immune responses. Promising preclinical data have been obtained for PMED DNA vaccines against some of the more challenging infectious disease vaccine targets, including HIV [50,52,54,69], smallpox virus [32,70,71], Ebola virus [31,44,72,73] and malaria [74-77]. Phase I clinical results for PMED DNA vaccines expressing antigens from hepatitis B virus, malaria and influenza provide an early indication of the safety of the technology, and the immunogenicity in humans. In particular, PMED DNA vaccination is notable for its ability to elicit not only antibody but also cellular responses in humans.

Preclinical and clinical feasibility of PMED DNA vaccination has been established; however, Phase II and III clinical studies will be required to demonstrate PMED DNA vaccine efficacy and/or effectiveness in humans, and to more comprehensively assess safety. The promising preclinical and early clinical results should be balanced with the need to establish practical dosing regimens. Although the dose of DNA required for PMED is lower that that required for intramuscular DNA vaccination, there is also a practical limit to the dose of DNA on gold that can be delivered to a small site on the skin. This limitation has resulted in some cases in the requirement for multiple dosings to achieve acceptable immunogenicity. Modifications to formulations or device parameters may be required to achieve consistent efficacy in humans using standard dosing regimens. Furthermore, the need to employ a device represents a complication in the manufacturing and distribution of PMED DNA vaccines that remains to be addressed. These limitations are less relevant in the case of vaccines for diseases for which there is no alternative prophylactic option, or for therapeutic vaccination against chronic infectious diseases or cancer. The ability of PMED DNA vaccines to elicit cellular immune responses provides a compelling basis for their use in such indications.

EPI protein vaccine delivery provides a mechanism to access this immune inductive site using conventional vaccine antigens. Early efforts have resulted in the development of stable dry powder adjuvanted and nonadjuvanted formulations of several antigens. Skin delivery may enable the use of potent adjuvants, which may have unacceptable toxicity when delivered parenterally. In addition to the delivery capability, these formulations should in theory enable room temperature storage of a wide range of antigens and adjuvants. Based on preclinical data in rodents, it was hypothesised that epidermal delivery would result in achieving equivalent or greater immunogenicity at a lower antigen dose. In nonhuman primates and in the initial clinical trial of EPI influenza vaccine, epidermal vaccination with an unadjuvanted formulation resulted in antibody titres that were equivalent to those elicited by parenteral immunisation [11,63]. These early results provide a promising start to formulation, regimen and device optimisation studies. Additional data showing the ability to elicit mucosal and cellular immune responses with protein antigens represent potential advantages of EPI vaccination. Further clinical trials with different antigens and formulations will be required to determine the most appropriate applications of EPI.

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