Safety and efficacy of transcutaneous vaccination using a patch with the live-attenuated measles vaccine in humans

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Abstract

Transcutaneous immunisation (TCI) using a skin patch is a non-invasive vaccination route relevant to mass vaccination against infectious diseases. This phase I/II clinical study, documents that TCI of human adult volunteers with the live-attenuated measles vaccine ROUVAX® is safe and poorly reactogenic. It promotes induction of measles-specific salivary IgA and a tendency to increased frequency of MV-specific IFN-γ-producing T cells. However, in contrast to the subcutaneous route, TCI failed to evoke neutralising MV-specific serum antibodies. Thus, alternative delivery methods and/or devices providing optimal uptake by skin DC should be considered for live-attenuated virus vaccines, such as the measles vaccine.

Keywords: Transcutaneous administration; Measles virus; Vaccines; IgA

1. Introduction

The skin is enriched in professional antigen-presenting dendritic cells (DC) including, epidermal Langerhans cells (LC), and dermal DC [1]. Transcutaneous immunisation (TCI) using a patch has recently emerged as a promising approach to target anti-infectious vaccines to DC. Indeed, studies in mice and human have demonstrated that TCI with bacterial toxins with adjuvant properties, such as cholera toxin (CT) or thermolabile enterotoxin from Escherichia coli (LT), triggered specific immunity to the toxin itself [2,3] or to a co-administered protein antigen [4–8] and was even able to trigger a protective mucosal IgA response [9]. Alternatively, TCI with proteins or peptides can lead to a Th2-biased response with production of specific IgE [10,11], or even a state of unresponsiveness [3,12,13], mediated by regulatory T cells [14,15]. Protective immunity by TCI with subunit vaccines thus appears to require the use of strong adjuvants such as bacterial toxins capable to bind to the GM1 ganglioside [2,16] and TLR ligands [17]. Mechanical disruption of the skin horny layer by tape stripping enhances skin permeability, favours access of the vaccine to epidermal LC and induces release by keratinocytes of proinflammatory cytokines inducing LC activation [18,19]. It is thus conceivable that TCI could be applicable to live-attenuated virus vaccines, either to enhanced the immunogenicity of existing vaccines or to
improve vaccine coverage in endemic regions with major socio-economic advantages.

Measles remains a major killer of infants in the third world, with an estimated half a million casualty each year, despite increased vaccine coverage and excellent efficacy of the current live-attenuated, subcutaneously administered vaccine [20,21]. It has been largely discussed that alternative needle-free vaccination routes would increase vaccine coverage and boost measles eradication efforts [22]. Moreover, transcutaneous immunisation (TCI) against measles during mass vaccination campaigns in endemic countries may have major socio-economical advantages. These include (i) safety, inasmuch as it would eliminate the possibility of cross-contamination of vaccinees with needles, as well as the risk of needle-stick injuries to healthcare workers and the risk resulting from unsafe sharps disposal practices to the community, and (ii) cost, because such a protocol would not require the intervention of specialised medical staff. Furthermore, this non-invasive and non-traumatic route of immunisation is also of interest both in developing areas and in developed countries for routine vaccination of infants and adolescents. Finally, both wild-type measles virus (MV) and vaccine MV strains infect DC including epidermal LC [23,24] and can induce signalling via TLR [25]. Since the live-attenuated MV vaccine delivered by skin puncture using bifurcated needles appeared to be poorly immunogenic [26], we reasoned that it could be more efficient when administered transcutaneously.

In this phase I/II clinical trial, we tested the acceptability, safety and immunogenicity of TCI using a patch with the monovalent live-attenuated measles vaccine ROUV AX® (Sanofi-Pasteur, Marcy L’Etoile, France), as follows. An 8-cm² surface of inner forearm skin was gently tape-stripped six times using a desquamation collector foil (Corneofix® R20, Monaderm, Monaco). A single drop of an estimated maximal volume of 5 µl of ROUV AX® (5 p.f.u.) or diluent alone was deposited on two sites of alcohol-swabbed inferior forearm skin and pricked vertically with a calibrated 1 mm lancet. Local and regional immediate reactions were monitored for 1 h after skin pricking. Positive controls for skin prick tests included histamine and codeine. Skin testing of delayed-type (T cell-mediated) hypersensitivity (DTH) reactions to the vaccine were carried out on dorsal skin both before and at day 10, 21 and 90 after vaccination. Peripheral blood mononuclear cells (PBMC) were collected before immunisation and at day 21 and 90 after vaccination. PBMC were isolated by centrifugation over Ficoll gradient and stored in liquid nitrogen. Serum samples were collected by centrifugation and stored at −20°C. Saliva samples were collected by mastication of a Salivette device (Sarsted, Orsay, France), centrifuged and stored at −80°C.

2. Materials and methods

2.1. Volunteer selection

Healthy volunteers aged 18–22 years with no history of measles infection and with a record of vaccination with live-attenuated measles in infancy were subjected to skin prick testing for assessment of immediate-type IgE-mediated hypersensitivity reactions to ROUVAX®. The ROUVAX vaccine was reconstituted with 0.1 ml diluent was then applied to the skin together with a single layer of sterile gauze and maintained under occlusion for 24 h with adhesive dressing.

2.2. Sample collection

Serum and saliva samples were collected before immunisation and at day 10, 21 and 90 after vaccination. Peripheral blood mononuclear cells (PBMC) were collected before immunisation and at day 21 and 90 after vaccination. PBMC were isolated by centrifugation over Ficoll gradient and stored in liquid nitrogen. Serum samples were collected by centrifugation and stored at −20°C. Saliva samples were collected by mastication of a Salivette device (Sarsted, Orsay, France), centrifuged and stored at −80°C.

2.3. Transcutaneous and subcutaneous immunisations

A group of 10 randomly selected volunteers were subjected to subcutaneous injection in the upper arm with a single dose of the monovalent live-attenuated measles vaccine ROUVAX® (Sanofi-Pasteur, Marcy L’Etoile, France), consisting in 10⁵ p.f.u. of Schwarz strain reconstituted in 0.5 ml diluent according to manufacturer’s instruction. Another group of 12 randomly selected volunteers received a transcutaneous immunisation with the ROUVAX® vaccine, as follows. An 8-cm² surface of inner forearm skin was monitored for 1 h after skin pricking. Positive controls for skin prick tests included histamine and codeine. Skin testing of delayed-type (T cell-mediated) hypersensitivity (DTH) reactions to the vaccine were carried out on dorsal skin both before and at day 10, 21 and 90 after vaccination. Peripheral blood mononuclear cells (PBMC) were collected before immunisation and at day 10, 21 and 90 after vaccination. PBMC were isolated by centrifugation over Ficoll gradient and stored in liquid nitrogen. Serum samples were collected by centrifugation and stored at −20°C. Saliva samples were collected by mastication of a Salivette device (Sarsted, Orsay, France), centrifuged and stored at −80°C.

2.4. Skin testing for immediate and delayed hypersensitivity reactions

Three months after vaccination, all individuals were subjected to skin prick testing for assessment of immediate-type IgE-mediated hypersensitivity reactions to ROUVAX®. The ROUVAX vaccine was reconstituted with 0.5 ml diluent according to manufacturer’s instructions. A single drop of an estimated maximal volume of 5 µl of ROUVAX® (5 p.f.u.) or diluent alone was deposited on two sites of alcohol-swabbed inferior forearm skin and pricked vertically with a calibrated 1 mm lancet. Local and regional immediate reactions were monitored for 1 h after skin pricking. Positive controls for skin prick tests included histamine and codeine. Skin testing of delayed-type (T cell-mediated) hypersensitivity (DTH) reactions to the vaccine were carried out on dorsal skin both before and at day 10, 21 and 90 after vaccination. Peripheral blood mononuclear cells (PBMC) were collected before immunisation and at day 10, 21 and 90 after vaccination. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Ficoll gradient and stored in liquid nitrogen. Serum samples were collected by centrifugation and stored at −20°C. Saliva samples were collected by mastication of a Salivette device (Sarsted, Orsay, France), centrifuged and stored at −80°C.
five randomly selected volunteers from each group were subjected to skin biopsies at the sites of patch tests with both vaccine and diluent.

2.5. Assessment of the acceptability of the procedure and of local and systemic reactions

For both immunisation groups, acceptability of the vaccination procedure was self-recorded by volunteers using an analogical scale ranging from 0 (no pain nor disagreement) to 10 (unacceptable). Local and regional clinical reactions were recorded by three daily clinical examinations following vaccination. Skin reactivity to vaccination was assessed by scoring of reactions including erythema, oedema, vesicles, scratching, itching and purpura. Each individual symptom was scored on a scale from 0 (no reaction) to 4 (severe reaction) and the general score was calculated as the sum of individual scores (maximum score: 24). Adverse systemic reactions such as fever, muscle pain, headache or asthenia were reported by the volunteers and recorded by clinicians.

2.6. Plaque-reduction neutralisation (PRN) assay

Serial twofold dilutions in culture medium of serum samples (100 μl) were incubated in microplates with 50 p.f.u. of attenuated measles virus (Schwarz strain, grown on VERO cells) for 1 h at 37°C. 2 × 10⁴ VERO cells in culture medium were added to the wells and cultured at 37°C for 4 days. Cells were then fixed with formalin and stained with methylene blue. Titres were determined as the dilution allowing the neutralisation of 50% of plaque-forming units (PRN units). Tests were standardised by parallel assessment of the international standard [27] (obtained from NIBSC, Salisbury, UK).

2.7. Measles-specific IgG ELISA titration

Measles virus (Schwarz strain)-infected VERO cells and mock-infected VERO cells were harvested and lysed in borate buffer, pH 9 (NaCl 120 mM, NaOH 24 mM, boric acid 50 mM). Infected and mock-infected cell lysates were coated onto separate Elisa plates (Nunc Maxisorp, Roskilde, Denmark). Serial dilutions of serum samples from volunteers and from the international standard were incubated onto plates. The assay was revealed by serial incubations of plates with biotin-coupled polyclonal Goat anti-Human IgG (Sigma, L’Isle d’Abeau, France), peroxidase-coupled streptavidin (Roche Diagnostics, Meylan, France) and O-phenylene diamine (OPD) (Sigma). Optical density was read using a SpectraMax reader (Molecular Devices, St. Grégoire, France). After subtraction of optical densities observed on mock-coated plates, samples were titrated against the international standard and expressed in mIU.

2.8. Measles-specific IgA titration in saliva

Total IgA levels in saliva were titrated as follows. ELISA plates were coated with polyclonal goat anti-human IgA antibody (Southern Biotech, Birmingham, AL, USA). Serial dilutions of saliva samples were incubated onto the plates, followed by incubation with peroxidase-coupled polyclonal goat anti-human antibody (Southern Biotech) and revelation by OPD. Human IgA immunoglobulin (Dade-Behring, Paris La Défense, France) was used as standard, and total IgA levels in samples were expressed in μg/ml. Measles-specific IgA levels were titrated by capture ELISA assay as follows. ELISA plates were coated with polyclonal goat anti-human IgA antibody (Southern Biotech). After incubation with diluted saliva samples, the assay was revealed by serial dilutions with borate-treated, MV-infected VERO cells or with mock-infected VERO cells, monoclonal mouse anti-measles Nucleoprotein (cl120), peroxidase-coupled polyclonal goat anti-mouse IgG (Southern Biotech) and OPD. After subtraction of optical densities observed on mock-coated plates, samples were titrated against an in-house positive saliva sample affected the titre of 100 arbitrary units (AU). Final sample titres were expressed as AU per μg total IgA protein (AU/μg IgA).

2.9. Measles-specific proliferation assay

PBMC (10⁵/well) were cultured for 5 days with 50 p.f.u. live VERO-grown measles virus (Schwarz strain) or mock-infected VERO cells. Proliferation was assessed by incorporation of ³H thymidine (0.5 μCi/well) during the last 18 h of culture. For each individual volunteer, the different time-points were tested in a single assay. Results of MV-specific proliferation determined for each PBMC sample (i.e., individual volunteer at a given time-point) are expressed as Δcpm: cpm with live MV-cpm with mock-virus.

2.10. MV-specific IFN-γ ELISPOT assay

ELISPOT plates (Millipore, France) were coated with monoclonal mouse anti-human IFN-γ antibody (Diaclone, Besançon, France). PBMC samples (10⁵ cells/well) were incubated for 36 h at 37°C with either 50 p.f.u. live measles virus (Schwarz strain) or mock-infected VERO cells and 2 U/well human IL-2 (Peprotech, Levallois Perret, France). Spots were revealed by incubation with biotin-coupled monoclonal mouse anti-human IFN-γ antibody (Diaclone), peroxidase-coupled streptavidin (Roche Diagnostics) and amino-ethyl-carbazole (Sigma). The different time-points for each individual were tested in a single assay. The number of IFN-γ spot forming cells (SFC) were calculated by subtracting the number of spots observed with mock-virus to the number of spots observed with live MV, for each individual volunteer at given time-points.
2.11. Statistical analysis

Mann Whitney’s nonparametric tests were performed using GraphPad Prism version 4 for Macintosh (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

3. Results

3.1. Safety and reactogenicity of TCI with ROUVAX®

TCI appeared significantly more acceptable than subcutaneous vaccination with acceptability scores <1 and of 2 (mild pain/discomfort), respectively \((P=0.04)\) (Fig. 1A). Clinical examination for local reactions at days 1, 3, 10 and 21 after vaccination showed only a very mild and transient oedema appearing at day 3 at the site of immunisation in 5/12 and 2/10 subjects of the transcutaneous immunisation group and the subcutaneous immunisation group, respectively (no statistical significance) (Fig. 1B). All observed oedemas were fully resolved by day 10. Mild systemic reactions were occasionally reported by the volunteers between day 1 and day 5 and consisted in mild fever (1 case in each immunisation groups), asthenia (2 cases in each group) or muscle ache (1 case, subcutaneous group). None of the volunteers required medical treatment. Skin prick tests for immediate-type hypersensitivity to ROUVAX® carried out at 3 months post vaccination were negative in all volunteers. As expected histamine and codeine gave a positive skin reaction, assessing for normal mast cell and endothelial cell reactivity (data not shown). No DTH reactions to ROUVAX® was observed. As immediate reactivity to skin prick testing is widely recognised as a mean to assess IgE-mediated vaccine allergy [28], this demonstrated that no MV-specific IgE responses were induced in vaccinees. Finally, no evidence for virus excretion was found as assessed by RT-PCR analysis of the cellular fraction of urine samples 3 and 10 days after vaccination (not shown). Therefore, in the current setting of previously immune individuals, transcutaneous vaccination appears as safe and acceptable as subcutaneous vaccination.

3.2. Rise in MV-specific neutralising antibodies in serum after subcutaneous, but not transcutaneous immunisation with ROUVAX®

As shown in Fig. 2A, subcutaneous immunisation with ROUVAX® increases the MV-specific serum IgG levels assessed by ELISA, compared to the levels observed before the trial. This increase was detectable by day 10 (although not significant) and was on average 10-fold higher at days 21 (median: 461; range: 11–6928 IU/ml) and 90 (median: 534; range: 27–1121) after immunisation as compared to pre-immunization level (median: 46; range: 1–362) \((P=0.005)\)
Fig. 3. Neutralising MV-specific antibodies production in serum before and after ROUVAX® immunisation: MV-specific neutralising antibodies present in serum were titrated before and at days 10, 21 and 90 after subcutaneous (A) or TCI immunisation (B) with ROUVAX® using an in vitro plaque-reduction neutralisation (PRN) test. Results are expressed PRN units calculated as the highest serum dilution able to neutralise 50% of measles plaque-forming units on Vero cells.

and \( P = 0.004 \), respectively) (Fig. 2A). Concomitantly, we observed a raise in circulating neutralizing antibodies as revealed by PRN titres at days 10 (median: 68; range: 30–102) day 21 (median: 102; range: 46–231) and 90 (median: 77; range: 46–102) after immunisation, compared to day 0 values (median: 30; range: 9–57) (\( P = 0.0052, P = 0.0003 \) and \( P = 0.003 \), respectively) (Fig. 3A). In contrast, no significant boost in MV-specific humoral immunity in the TCI group could be demonstrated by either ELISA titration (Fig. 2B) or PRN assay (Fig. 3B). Thus, subcutaneous but not TCI vaccination on previously immune individuals selected on the basis of their low levels of circulating antibodies is able to induce a significant and sustained increase in humoral immunity to MV.

3.3. TCI with ROUVAX increases MV-specific salivary IgA levels

Titration of measles-specific IgA compared to total IgA levels was carried out in saliva before and at days 10, 21 and 90 after ROUVAX® immunisation via the subcutaneous route and after TCI, by a sandwich Elisa method allowing for the detection of nucleoprotein-specific antibodies. The levels of MV-specific salivary IgA were low to undetectable in each patient before the vaccination protocol. No increase in MV-specific IgA levels was observed in volunteers at any time point after subcutaneous immunisation. Conversely, volunteers who received ROUVAX® transcutaneously transiently increased their IgA levels in saliva an average of 5 times at day 21 after immunisation (median: 0.5; range:0.03–10.9), compared to levels observed before immunisation (median: 0.09; range:0.01–2.8) (\( P = 0.049 \)). As expected for mucosal IgA responses, the levels of MV-specific salivary IgA returned to pre-immunisation levels by day 90 (Fig. 4).

3.4. MV-specific T cell responses after TCI and subcutaneous ROUVAX® vaccination

The MV-specific cellular immune response in PBMC was analyzed before and on days 21 and 90 after vaccination using cell proliferation and IFN-\( \gamma \) ELISPOT assays. Quantitative analysis on the whole population revealed that MV-specific cell proliferation tended to rise at day 21 post-vaccination in the subcutaneous vaccine recipients (median: 2925; range:1352–8358) as compared to day 0 values (median: 2717; range:1188–4299), although this difference was statistically non significant (\( P = 0.06 \), Fig. 5A, upper panel). This parameter hardly seemed affected in transcutaneous vaccine recipients (Fig. 5A, lower panel). In contrast, while there was clearly no increase of MV-specific IFN-\( \gamma \) production by PBMC after subcutaneous vaccination (Fig. 5B, upper panel), there was an apparent rise in the frequency of MV-specific IFN-\( \gamma \) producing cells after tran-
Fig. 5. Cellular response to ROUVAX®. MV-specific T cell-mediated responses of PBMC from each individual volunteer on days 0, 21 and 90 after ROUVAX® vaccination via the subcutaneous (upper panels) and the transcutaneous (lower panels) routes. (A) MV-specific proliferation of PBMC is expressed as Δcpm, calculated by subtracting cpm in mock-virus-stimulated cultures to cpm in MV-stimulated cultures. (B) Frequency of MV-specific IFNγ SFC in 1×10^6 PBMC determined by Elispot, is calculated by subtracting the number of IFN-γ SFC/1×10^6 PBMC cultured with mock-virus to the number of IFN-γ SFC/1×10^6 PBMC cultured with MV. For each individual, PBMC at the different time points were tested in the same assay.

scutaneous vaccination, with 2.3-fold increase of the mean at day 90 post vaccination (Fig. 5B, lower panel). However, due to important differences between individuals in given groups and small sample size, this rise did not appear statistically significant. Nevertheless, as shown in Table 1, qualitative analysis of the IFN-γ Elispot data also demonstrated a trend toward higher responses in the TCI group as compared to the subcutaneous group, suggesting that TCI might induce a Th1-type bias in MV-specific immunity.

4. Discussion

This phase I/II clinical study provides a proof of principle of acceptability, safety and immunogenicity of TCI with ROUVAX® using a patch in pre-immune healthy volunteers. The level of pain and/or discomfort recorded is inferior for the transcutaneous procedure as compared to the subcutaneous procedure. Interestingly, TCI with live-attenuated MV appeared as safe as the subcutaneous route in view of lack of major clinical local and systemic reaction and absence of virus secretion. In both groups of vaccines, skin prick tests to the vaccine were always negative, despite functional skin reactivity to positive controls (codeine and histamine), indicating lack of induction of specific IgE [28]. These data thus provide a proof of principle that the advantage of this needle-free procedure in terms of acceptability and safety can be extended to live-attenuated vaccines.

The study was conducted in MV pre-immune volunteers who had been vaccinated against measles in infancy. The MV-specific antibody response was enhanced in the control group of recipients vaccinated with ROUVAX® subcutaneously, as revealed by the rise in both MV-specific serum IgG and seroneutralising antibodies. This confirms that a vaccination boost can be measured in individuals with low levels of pre-existing MV-specific antibodies [29]. However, no such boost

### Table 1

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<th>Non-responders</th>
<th>Low responders</th>
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<tr>
<td>Subcutaneous</td>
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<td>Transcutaneous</td>
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Non-responders: 1 ≤ fold-increase in IFN-γ SFC < 1.5; low responders: 1.5 ≤ fold-increase in IFN-γ SFC < 4; high responders: 4 ≤ fold-increase in IFN-γ SFC.
was observed in the group of volunteers vaccinated with ROUVAX® by the transcutaneous route.

Alternatively, TCI – but not subcutaneous – vaccination increased the levels of MV-specific salivary IgA, indicating that mucosal immunity can be induced by TCI with a live vaccine. This is reminiscent of previous studies showing that TCI with CT induces secretory IgA in the respiratory tract and protects against a lethal nasal challenge with CT [9]. According to the concept of compartmentalisation of the common mucosal-associated immune system (MALT) [30], homing of antigen-specific IgA B cells to mucosal sites distinct from the initial site of immunisation can generate secretory IgA in various exocrine secretions. Thus, induction of MV-specific salivary IgA by TCI suggests that IgA may be also produced in the upper respiratory tract (considered as the natural site of MV entry, as well as its main replication site for the generation of contagious fluids) and therefore may contribute to protective immunity against measles. In addition, presence of MV-specific IgA in TCI recipients provides a proof that the vaccine delivery procedure by application of an occlusive patch onto tape-striped skin has allowed vaccine penetration and accessibility to the immune system.

Reasons for the discrepancies in humoral immune responses observed between transcutaneously or subcutaneously vaccinated individuals are unclear. It is assumed that the efficacy of TCI can be attributed to by the targeting of the vaccine to Langerhans cells (LC), because these resident epithelial dendritic cells (DC) have been long recognised to be crucial for induction of immune responses to antigens penetrating via the skin. However, current data obtained in several animal models have raised substantial controversy about this dogma, and the paradigm that antigen processing and presentation by LC migrating from epithelial tissues to local lymph node results in priming of antigen-specific T cells and protective immunity likely needs to be revisited. For instance, during infection of mouse epidermis by herpes simplex virus, virus-specific T cell priming is not accomplished by LC but by lymph-node resident CD8α-positive DC [31]. Likewise, following genital infection by herpes simplex in mice, Th1-type cells are primed in local lymph nodes by DC previously recruited in the submucosa at the site of infection and not by resident vaginal LC [32]. Alternatively, TCI using protein without adjuvant results in a Th2-biased immune response with regulatory T cells [10,14]. These data could be reconciled by the assumption that LC are conditioned by the skin microenvironment, rich in TGF-β and IL-10 which are crucially implicated in development and function of regulatory T cells and inhibition of Th1 responses [33]. This may explain why TCI favours the production of mucosal IgA, since DC under the influence of TGF-β and IL-10 promote IgA switch [34,35].

In spite of a substantial boost of MV-specific IgG levels in all volunteers receiving subcutaneous ROUVAX®, we observed little change in MV-specific T cell proliferation or IFN-γ production in this immunisation group. These generally low MV-specific T cell-mediated responses, demonstrated after in vitro restimulation of PBMC with MV, were also undetectable in HLA-A0201-typed volunteers’ PBMC upon restimulation with a cocktail of published and specially designed class I peptides derived from MV-C, -HA, -NP or -F proteins [36] (not shown). Moreover, no skin DTH response was observed in any of the volunteers 3 months post vaccination, regardless of the vaccination route and of the skin challenge route (i.e., intradermal or epicutaneous). This could be attributed either to the dose of the vaccine and/or the pre-immune status of the volunteers, in which a boost in cellular immunity might be less easily detectable than a boost in humoral immunity. In addition, it cannot be formally excluded that live MV used for in vitro restimulation may have simultaneously caused T cell immune suppression and/or apoptosis [23,37], that could have masked MV-specific proliferation and cytokine production. Despite this caveat, it should be emphasised that 9 out of 12 of TCI recipients versus only 3 out of 9 individuals of the subcutaneous group exhibited a detectable increase of MV-specific IFN-γ production on days 21 or 90 after vaccination. Although this observation did not appear statistically significant given the high inter-individual variability and the reduced number of volunteers, this suggests that TCI might be more efficient at inducing a Th1-type response than subcutaneous vaccination, possibly via concomitant uptake by dermal DC. This hypothesis is supported by recent observations that intradermal delivery of CT [38], as well as intradermal or buccal immunisation with either CpG, poly (I:C), flagellin, the hapten DNFB or recombinant measles Nucleoprotein can induce priming of class I-restricted CD8+ CTL to a co-administered protein [39]. We also demonstrated that newly recruited DC derived from circulating monocytes (rather than skin-resident DC), are the antigen-presenting cells responsible for the priming of CD8+ CTL [39].

Along these lines, TCI using a subunit vaccine is more effective than systemic or oral immunization for the generation of mucosal immunity and not only secretory IgA but also mucosal CTL and may contribute to protective immunity against measles as well [9,17,40,12]. Since, induction of protective cytotoxic CD8+ T cells would be invaluable for an anti-viral vaccine, strategies to broaden its protective efficacy by co-administration of appropriate adjuvants, may be relevant for TCI with live vaccines against measles or other respiratory virus. In this respect, CpG ODN increase the protective efficacy of modified vaccinia Ankara (MVA) by enhancing the CD8 CTL response in the lung, even in the absence of CD4+ T cells, and more efficiently via the nasal route [41]. Although CpG ODN also exhibit CD8 adjuvant properties when topically applied onto the skin, it’s ability to enhance delayed-type skin hypersensitivity to a co-administered antigen, suggest that it may evoke unacceptable skin reactogenicity and immune side-effects such as T cell-mediated skin diseases [42].

Our study provided the first proof of principle that transcutaneous vaccination with a live-attenuated virus vaccine can promote induction of a virus-specific IgA response in secre-
tory fluids. Since MV is not a bona fide respiratory virus, it remains to be determined whether our findings also apply to other virus vaccines, which unlike MV do not infect the DCs. Indeed, induction of secretory IgA in the respiratory tract by TCI would especially be of interest for live-attenuated vac-
cines against true respiratory virus infections (i.e., influenza
virus, respiratory syncytial virus, human metapneumovirus
or parainfluenzavirus).

Alternative delivery routes are being evaluated for immu-

nisation with measles vaccine. The aerosol route with a
delivery device has shown significant efficacy [43], although
a potential drawback to this method is the difficulty to cali-
brate the immunisation dose, particularly for infants. While
immunisation using a skin patch could be simpler than using
a nebuliser, TCI’s inability to boost serum antibodies, at least
with the settings used in this study, precludes its use for
mass vaccination. It may be proposed that vaccination with
live-attenuated virus could benefit from alternative skin deliv-
ery methods such as intradermal delivery, favouring access
both epidermal LC, to trigger mucosal IgA responses, and to
resident or newly recruited dermal DC, to allow for CD8+
CTL induction. This could be achieved by new intradermal
delivery devices which (unlike bifurcated needles) target the
dermo-epidermal junction, appear efficient with subunit vac-
cines [44] and can be adapted for live-attenuated vaccines.

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