Title

A Microneedle Patch for Measles and Rubella Vaccination Is Immunogenic and Protective in Infant Rhesus Macaques.

Journal

The Journal of Infectious Diseases, 218(1)

Authors

Joyce, Jessica

Carroll, Timothy

Collins, Marcus

et al.

Publication Date

2018-06-05

DOI

10.1093/infdis/jiy139

Peer reviewed

The Journal of Infectious Diseases

MAJOR ARTICLE

A Microneedle Patch for Measles and Rubella Vaccination Is Immunogenic and Protective in Infant Rhesus Macaques

Jessica C. Joyce,[1,a] Timothy D. Carroll,[2] Marcus L. Collins,[3] Min-hsin Chen,[3] Linda Fritts,[2] Joseph C. Dutra,[2] Tracy L. Rourke,[2] James L. Goodson,[3] Michael B. McChesney,[2] Mark R. Prausnitz,[1,4] and Paul A. Rota[3]

[1]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta; [2]Center for Comparative Medicine, and California National Primate Research Center, University of California, Davis, CA; and [3]Centers for Disease Control and Prevention, Atlanta, GA and [4]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA

Background. New methods to increase measles and rubella (MR) vaccination coverage are needed to achieve global and regional MR elimination goals.

Methods. Here, we developed microneedle (MN) patches designed to administer MR vaccine by minimally trained personnel, leave no biohazardous sharps waste, remove the need for vaccine reconstitution, and provide thermostability outside the cold chain. This study evaluated the immunogenicity of MN patches delivering MR vaccine to infant rhesus macaques.

Results. Protective titers of measles neutralizing antibodies (>120 mIU/mL) were detected in 100% of macaques in the MN group and 75% of macaques in the subcutaneous (SC) injection group. Rubella neutralizing antibody titers were >10 IU/mL for all groups. All macaques in the MN group were protected from challenge with wild-type measles virus, whereas 75% were protected in the SC group. However, vaccination by the MN or SC route was unable to generate protective immune responses to measles in infant macaques pretreated with measles immunoglobulin to simulate maternal antibody.

Conclusions. These results show, for the first time, that MR vaccine delivered by MN patch generated protective titers of neutralizing antibodies to both measles and rubella in infant rhesus macaques and afforded complete protection from measles virus challenge.

Keywords. microneedle patch; skin vaccination; measles; rubella; transdermal delivery.

Measles and rubella have been controlled in many parts of the world by achieving high levels of coverage with measles and rubella (MR) vaccine [1]. The World Health Assembly established vaccination coverage targets in 2010 of ≥90% nationally and ≥80% in every district and, in 2012, endorsed the Global Vaccine Action Plan that set a global goal for elimination of MR in 5 of the 6 World Health Organization (WHO) regions by 2020 [2]. By 2013, countries in all 6 regions adopted measles elimination goals, and 3 regions established goals for elimination of rubella and congenital rubella syndrome. However, in 2015, global coverage with the first dose of measles-containing vaccine remained at 85%, where it has plateaued since 2009, and coverage with the first dose of rubella-containing vaccine was only 46%. In 2015, measles caused an estimated 135 000 child deaths, and an estimated 100 000 children were born with congenital rubella syndrome [3, 4].

The currently available MR vaccines have characteristics that cause many logistical challenges for achieving high vaccination coverage. They must be delivered by subcutaneous (SC) injection, require strict adherence to the cold chain, require reconstitution with diluent, must be discarded within 6 hours after reconstitution, and generate hazardous waste that requires safe disposal [5, 6]. Reuse and mishandling of syringes and needles can result in sharps injuries and transmission of blood-borne pathogens. Human error in reconstitution with incorrect diluents has led to serious adverse events including deaths. Global partners have called for investments in research and innovation to overcome these challenges, including expeditious development and licensure of a microneedle (MN) patch for MR vaccination [7]. For example, MR vaccination by MN patch would simplify logistics in routine immunization clinics and facilitate implementation of house-to-house vaccination campaigns, a strategy that was key for success in both the polio and smallpox eradication programs [8, 9].

MN patches are a promising novel method for administering MR vaccine [5, 6, 10, 11]. The patches consist of an array of solid MNs, each several hundred micrometers in length. These MNs are made of vaccine encapsulated in water-soluble polymers, sugars, and other excipients in a formulation that can painlessly pierce the skin, where the MNs dissolve to release the vaccine [12–14]. MN patches are small in size (eg, 1 cm^2) and mass (eg, 5 g), do not require reconstitution medium, have a

Received 29 January 2018; editorial decision 5 March 2018; accepted 13 March 2018; published online April 26, 2018.

[a]Present affiliation: GlaxoSmithKline, Collegeville, PA.

Presented in part: American Society for Virology, Madison, Wisconsin, 24–28 June 2017; Measles Mini-symposium, Mayo Clinic Rochester, Minnesota, 23–24 June 2017; American Association of Pharmaceutical Sciences Biotechnology Conference, San Diego, California, 1–3 May 2017.

Correspondence: P. Rota, PhD, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS-C22, Atlanta, GA 30033 (prota@cdc.gov).

The Journal of Infectious Diseases® 2018;218:124–32

DOI: 10.1093/infdis/jiy139

single-dose presentation, and can be designed for administration by hand without the need for an applicator. Because the vaccine is encased in stabilizing excipients, MNs can be stored at ambient or elevated temperatures for long periods without loss of vaccine potency [15–18]. Patches can be administered by minimally trained personnel or even self-administered and are strongly preferred by patients over hypodermic injection [19–21]. Because MNs dissolve in the skin, used patches cannot be reused and pose no risk for needle-stick injuries [5].

In our previous work, MN patches loaded with measles vaccine were immunogenic in cotton rats and juvenile rhesus macaques and exhibited no loss of potency when stored at 25°C for up to 4 months [15, 22]. Here, we report the formulation of an MN patch containing MR vaccine and an assessment of its immunogenicity in infant rhesus macaques. As MN patches deliver vaccines to the skin, an organ rich in resident antigen-presenting cells, and induce robust immune responses [23–27], we also evaluated the possibility of MR MN patch vaccination to overcome maternal antibody inhibition.

MATERIALS AND METHODS

Vaccines

The Edmonston–Zagreb vaccine strain for measles and the RA-27 rubella vaccine were obtained from stocks at the Centers for Disease Control and Prevention (CDC); these strains are the most commonly used MR vaccine in the global program. Vaccines were passaged in Vero cells (American Type Culture Collection [ATCC] CCL-81, Manassas, Virginia) maintained in Dulbecco's modified eagle medium (DMEM, Gibco, Grand Island, New York) and 2% fetal bovine serum (FBS; Gibco). Infected cells were harvested, freeze-thawed, and subjected to low-speed centrifugation to separate the virus from cellular debris. Vaccine aliquots were stored at –80°C [22].

Production of MN Patches

MN patches were prepared as previously described [15, 16, 28]. In brief, measles vaccine, rubella vaccine, and an excipient solution of sucrose, threonine, and carboxymethylcellulose (Sigma-Aldrich, St Louis, Missouri) in potassium phosphate buffer (pH 7.5) were mixed and placed on a polydimethyl siloxane micromold with vacuum. The vaccine solution was allowed to dry into the tips of the MN cavities, and residual material on the micromold surface was removed via tape-stripping. A solution of polyvinyl alcohol (Acros Organics, Geel, Belgium), sucrose, and deionized water was added. After 2 days in a desiccator at room temperature (20°C –25°C), MN patches were demolded and stored desiccated at room temperature until use. To measure the efficiency of delivery, MN patches were inserted into pig skin ex vivo for 20 minutes to allow complete dissolution of the MNs. The skin was stained with gentian violet (Humco, Texarkana, Texas) to visualize puncture holes. Viral titers measured before and after insertion were used to estimate the delivered dose.

To measure the stability of MN patches, patches were sealed in aluminum foil pouches (Oliver-Tolas Healthcare, Grand Rapids, Michigan) with desiccant. Pouches were stored in an environmental test chamber at 40°C (Caron, Marietta, Ohio) for 1 month. At various time points, patches were removed from the chamber, and viral titers were measured.

To determine the titer of the measles vaccine, 10-fold dilutions of vaccine were prepared and inoculated onto 48-well tissue culture plates containing monolayers of Vero cells (ATCC, CCL-81) in DMEM with 2% FBS. Six wells were inoculated with each dilution and, after 5 days, each well was observed for evidence of viral cytotoxic effect by staining with 0.05% crystal violet and 1% formalin. To determine rubella vaccine titer, vaccine was incubated for 1 hour with antimeasles immunoglobulin G (IgG) (EMD Millipore, Billerica, Massachusetts) at 37°C to inhibit measles vaccine infection. Ten-fold dilutions of the vaccine-measles IgG solution were prepared and inoculated onto Vero cell monolayers as described above. After 5 days, cells were fixed with 10% methanol, and immunostaining was used to visualize infected foci [29]. Titers expressed as 50% tissue culture infectious dose ($TCID_{50}$) were calculated using the Spearman and Karber algorithm [30].

Vaccination and Challenge of Nonhuman Primates

Colony-bred infant macaques used in the study were housed at the California National Primate Research Center in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care International Standards. The Institutional Animal Use and Care Committee of the University of California, Davis, the Georgia Institute of Technology, and the CDC approved these experiments. Infant macaques were cohoused with measles and rubella–seronegative dames and weaned 42 days (± 5 days) prior to challenge. Animal vital signs were regularly monitored. For blood collection and virus inoculation, animals were anesthetized with 10 mg/kg ketamine hydrochloride (Parke-Davis, Detroit, Michigan) injected intramuscularly.

Sixteen infant macaques (18–49 days old; mean body weight, 0.5 kg) were assigned to 1 of 4 experimental groups (Supplementary Table 1). As previously described [31], 4 mL of measles immunoglobulin (MiG) were administered by the intraperitoneal route to 2 groups at a mean of 32 days (± 9.6 days) after birth and 48 hours prior to vaccination. MiG-treated and -untreated groups were administered MR vaccination by either SC injection (3.2×10^3 $TCID_{50}$ of measles vaccine and 3.2×10^3 $TCID_{50}$ of rubella vaccine in 0.5 mL phosphate-buffered saline) or MN patch (4.3×10^3 $TCID_{50}$ of measles vaccine and 3.6×10^3 $TCID_{50}$ of rubella vaccine). For MN vaccination, patches were pressed onto the skin of the inner thigh, and gentle pressure was applied for 30 seconds. Patches were then left on the skin for 15 minutes to allow for MN dissolution. Serum samples were collected every 2 weeks postvaccination

(PV), except at 8, 16, and 26 weeks PV, when heparinized blood samples were collected. At week 28 PV (week 0 postchallenge [PC]), all vaccinated infant macaques in addition to an unimmunized group of macaques (mean age, 365 days) were challenged by intranasal inoculation with wild-type measles virus 1×10^5 $TCID_{50}$/mL. The derivation of the viral stock for the challenge is described in the Supplementary Methods. In methods previously described [31], nasopharyngeal washes and heparinized blood samples were collected on 0, 1, 2, 3, 4, 5, 8, and 12 weeks PC. Peripheral blood mononuclear cells (PBMCs) were obtained from all macaques on the day of challenge (day 0), and 7, 14, and 21 days after challenge.

Following challenge, measles viremia was measured by co-culturing dilutions of PBMCs with Raji cells as previously described [32]. In addition, the copy number of measles RNA in cryopreserved PBMCs was measured by a reverse-transcription quantitative polymerase chain reaction assay (RT-qPCR). In brief, 10^5 PBMCs were thawed and centrifuged at 1500*g* for 5 minutes, and RNA was extracted by using a RNeasy Micro Kit (Qiagen, Hilden, Germany). Complementary DNA was prepared using random hexamer primers (ThermoFisher, Waltham, Massachusetts) and SuperScript III reverse transcriptase (ThermoFisher). Copy numbers of the RNA coding for the measles nucleoprotein (N) gene were measured by RT-qPCR as previously described [33] using a standard curve produced with a purified amplicon containing a fragment of the N gene. A constitutively expressed reference gene, GAPDH, was included to control for cellular RNA input and quality of the RNA extraction.

Serologic Methods

Neutralizing antibody titers to measles were determined using the standard plaque reduction neutralization (PRN) assay [34], and titers were calculated based on Third WHO International Standard Reference Serum (97/648). Neutralizing antibodies to rubella were measured using a focus-reduction assay as described previously [29]. A value of 1 IU/mL was assigned to all serum samples with titer <5 IU/mL.

RESULTS

Fabrication of Microneedle Patches

The MN patches used in this study consisted of an array of 100 MNs in a 10 × 10 grid of approximately 1 cm^2 mounted on a backing structure to facilitate handling (Figure 1A). The MNs were solid, conical structures made of water-soluble excipients and contained approximately 4000 $TCID_{50}$ of measles and rubella vaccine (Figure 1B). The MN patches could be pressed onto the skin and, upon penetration into skin, the MNs dissolved, leaving behind only the base structure on which they were mounted (Figure 1B). The MN patches were thermostable (Figure 1C); when stored for up to 1 month at 40°C, there was no significant loss of vaccine potency (analysis of variance, $P > .1$), which exceeds the WHO requirement for stability at 37°C for 1 week [35]. For future storage and use in routine immunization clinics and mass vaccination campaigns, a packaging concept was developed to package MN patches on blister trays housed in cardboard boxes (Figure 1D). In this configuration, each single-dose MN patch required 10 cm^3 of packaged volume and 4 g of packaged weight.

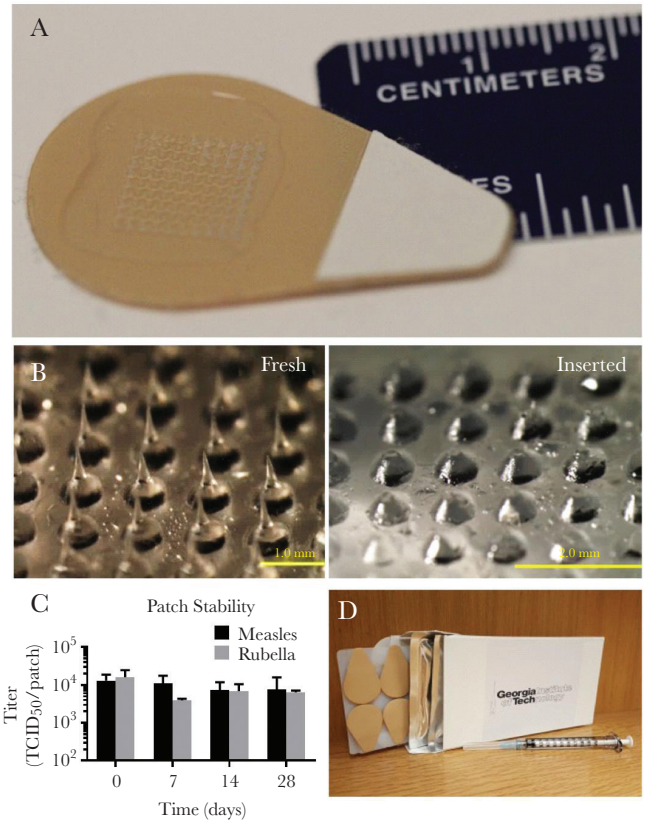

Figure 1. Microneedle (MN) patches consist of micrometer-scale projections encasing measles and rubella (MR) vaccine in water-soluble excipients (*A*). The MN array is 1 cm^2, and the entire patch is 3.5 × 2.2 cm in size (*B*, left image). The MN patches are 700 µm long and, once inserted, the needles dissolve completely, leaving only a blunt base structure (*B*, right image). MN patches were stored at 40°C for up to 1 month without significant loss in vaccine activity (*C*). Data show mean titer ± standard deviation (n = 4 replicates). A box containing 50 doses of MR vaccine is shown next to a 1-mL syringe (*D*). Abbreviation: $TCID_{50}$, 50% tissue culture infectious dose.

Vaccination With a Microneedle Patch

Rhesus macaques, born to measles-naive mothers, were vaccinated at 3–4 weeks of age (Supplementary Table 1). MN patches were manually applied to the inner thigh of the macaques and left in place for 15 minutes to allow dissolution (Figure 2A). Immediately after removal, an array of faint puncture marks corresponding to the MN puncture sites could be seen on the skin (Figure 2B), but they were not visible 1 hour after vaccination. Additionally, very mild, transient erythema, but no edema or bleeding, was observed; no adverse effects were noted at any point during the study.

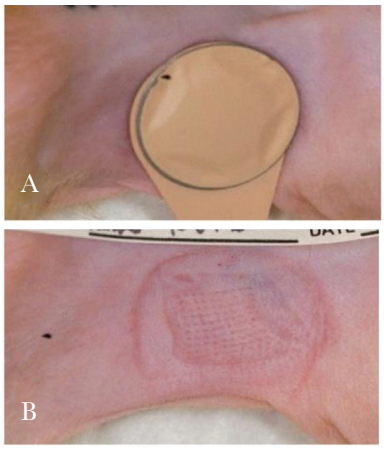

Figure 2. *A*, Microneedle patches were applied to the inner thigh of infant rhesus macaques. *B*, Immediately after removal, a faint grid pattern can be seen on the leg due to tiny punctures from each microneedle. After a few minutes, this grid pattern disappeared (not shown). Very mild, transient erythema, but no edema or bleeding, was observed following removal of the patch.

Titers of neutralizing antibody to measles increased after vaccination, and at day 42, 100% of infant rhesus macaques in the MN patch group had protective titers of >120 mIU/mL, while 50% of rhesus macaques in the SC group had protective titers (Figure 3; Supplementary Table 1). One of the macaques with low neutralizing antibody titers on day 42 had a titer >120 mIU/mL on the day of challenge (Figure 4C). The failure of some of the macaques in the SC group to seroconvert is unclear, though previous studies have shown that 75%–80% of rhesus macaques vaccinated by the SC route generated protective titers [36, 37]. Overall, these data indicated that vaccination by MN patch induced a neutralizing antibody response that was at least equivalent to the response induced by SC injection in naive infant macaques. All infant macaques had protective titers of neutralizing antibodies to rubella on day 42 (Figure 3), and all titers were substantially above a protective titer of 10 IU/mL (Figure 3). In general, rubella neutralizing antibody titers were higher after MN patch vaccination compared with SC injection, but these differences were not significant.

Measles Challenge

Approximately 7 months after vaccination, all vaccinated macaques, as well as 4 unimmunized controls, were challenged intranasally with wild-type measles virus. All vaccinated infant macaques showed no clinical signs of infection such as coughing or rash, except for the 1 macaque in the SC group that failed to seroconvert (Supplementary Table 1). Infant macaques vaccinated with MN patches had no detectable viremia as measured by detection of infectious measles virus or viral RNA in PBMCs 7 and 17 days after challenge (Figure 4). Other than the infant rhesus macaque that failed to seroconvert, all macaques in the SC injection group had no detectable infectious measles virus in their PBMCs, though a low level of measles RNA was detected in 1 macaque (Figure 4). Therefore, vaccination of infant macaques with an MN patch induced a protective immune response, which was at least as effective as the protection provided by SC injection.

All macaques in the MN group and 75% of macaques in the SC group had protective titers on the day of challenge, and by day 14 after challenge, the high titers of neutralizing antibodies were detected in all macaques (Figure 4). Except for the macaque from the SC group that failed to seroconvert to vaccination, there was no measurable immunoglobulin M (IgM) response in the vaccinated macaques following challenge.

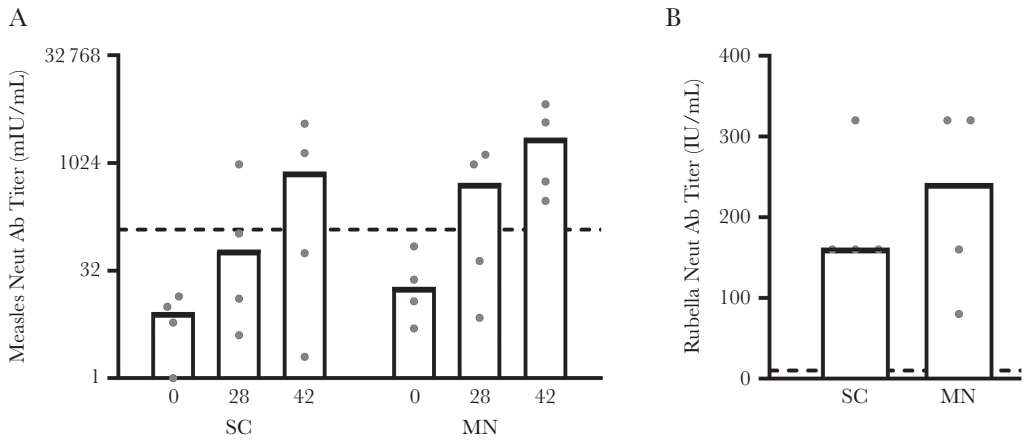

Figure 3. Neutralizing antibody (Neut Ab) titers to measles and rubella following vaccination of infant rhesus macaques with a microneedle (MN) patch or subcutaneous (SC) injection. The bars represent the median Neut Ab titer of each group; dots represent titers for each rhesus macaque. Measles-specific Neut Abs (*A*) were measured on days 0, 28, and 42. The dotted line indicates the minimum protective titer of 120 mIU/mL. Rubella-specific Neut Abs (*B*) were measured on day 42. Dashed line indicates the protective titer of 10 IU/mL.

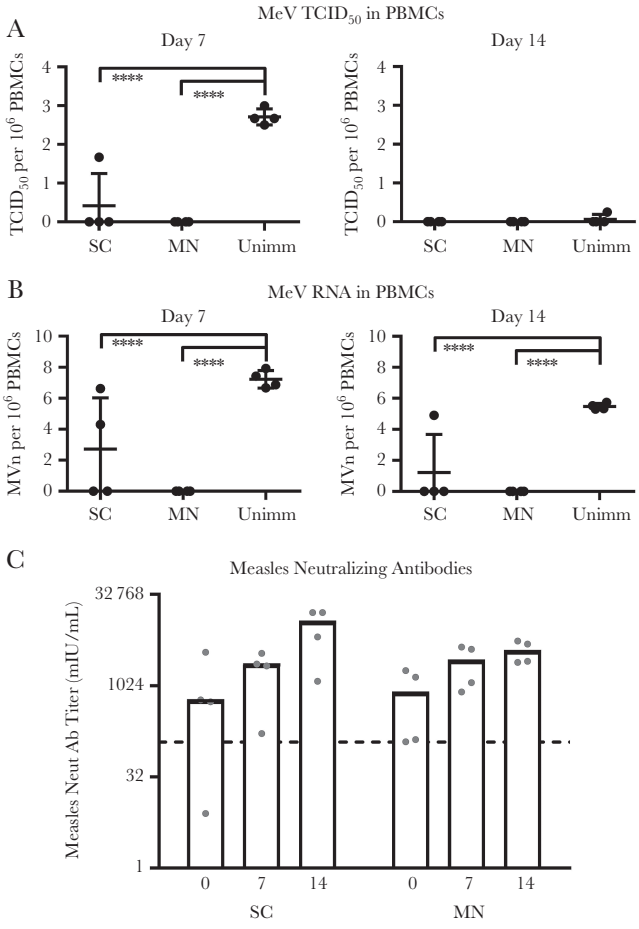

Figure 4. Vaccination with a measles and rubella vaccine by microneedle patch or by subcutaneous injection protects infant rhesus macaques from challenge with wild-type measles virus. At 202–216 days (mean, 212 days after vaccination; mean age, 244 days) after vaccination, all vaccinated macaques and 4 naive controls (mean age, 365 days, unimmunized) were challenged with 1×10^5 50% tissue culture infectious dose ($TCID_{50}$) by the intranasal route. Peripheral blood mononuclear cells (PBMCs) were obtained from all macaques on the day of challenge (day 0), as well as days 7, 14, and 21 after challenge. Measles viremia was measured by co-culturing dilutions of PBMCs with Raji cells, extracting RNA from PBMCs, and conducting reverse-transcription quantitative polymerase chain reaction to determine the number of copies of measles RNA in each sample. Results for each rhesus macaque are expressed as $\log_{10}$ $TCID_{50}$ of measles virus (*A*) or $\log_{10}$ copies of measles RNA (*B*) per 10^6 PBMCs (lines indicate mean and standard deviation). All macaques vaccinated with the microneedle patch and 3 of 4 macaques vaccinated by subcutaneous injection were protected from challenge. *C*, Neutralizing antibody responses to measles virus on day of challenge and following challenge. The bars represent the median neutralizing antibody titer of each group; dots represent titers for each rhesus macaque. ****P <.0001. Abbreviations: MeV, measles virus; MN, microneedle; MVn, measles virus N RNA; Neut Ab, neutralizing antibodies; PBMCs, peripheral blood mononuclear cells; SC, subcutaneous; $TCID_{50}$, 50% tissue culture infectious dose; Unimm, unimmunized.

Vaccination With a Microneedle Patch in the Presence of Measles Immunoglobulin

Maternal antibodies can interfere with the infant immune response to MR vaccination [38]. Because maternal antibodies begin to wane as early as 2–4 months of age, infants become susceptible to measles and rubella before age eligibility for vaccination, meaning that a novel MR vaccine that could illicit a protective immune response when administered within the first 6 months of life would be highly beneficial. Therefore, we tested the ability of the MR microneedle vaccine to generate an immune response in the presence of MiG.

Infant macaques vaccinated by MN patch or SC injection after receiving MiG failed to generate protective neutralizing antibody responses to measles by day 42 (Figure 5; Supplementary Table 1). In these groups, the input MiG was still detected at day 42 (Figure 5). Previous studies showed that MiG could be detected in infant macaques for up to 8–10 weeks after administration [36, 39], which is consistent with our data (Figure 5). The presence of measles-specific MiG had no effect on rubella vaccination.

All infant rhesus macaques vaccinated in the presence of MiG displayed clinical signs of infection that were comparable to those of the unimmunized controls, and all macaques had viremia as measured by detection of infectious measles virus and measles RNA in PBMCs at days 7 and 14 after challenge (Supplementary Table 1). The viral titers and copy number of measles RNA in macaques vaccinated in the presence of MiG were like those of the unimmunized controls (Figure 4; Supplementary Table 1).

All the rhesus macaques in the MiG treatment group were seronegative for measles on the day of challenge approximately 30 weeks after MiG administration (Figure 5); however, by day 14 after challenge, protective titers of neutralizing antibody titers were detected in all animals. All macaques vaccinated in the presence of MiG had an IgM response by day 14 after challenge, suggestive of a primary immune response to measles (Figure 5).

DISCUSSION

The vaccines to prevent measles and rubella are among the safest and most efficacious vaccines in use today. However, delivery of MR vaccine by SC injection presents logistical challenges, limiting efforts to reach the high vaccination coverage needed to achieve global and regional MR elimination goals. The Global Vaccine and Immunization Research Forum in 2016 emphasized the critical need for novel vaccine delivery tools that could increase coverage [40]. Here, we evaluated the immunogenicity of an MR vaccine delivered by a novel delivery method, the MN patch, in infant rhesus macaques.

MN patches have been shown to be effective for delivery of a variety of vaccines [5, 6, 10, 11], including prior studies of measles vaccination of cotton rats and juvenile rhesus macaques [15, 22] and a recent human clinical trial of influenza vaccination [21]. Here, we report successful delivery of 2 live attenuated vaccines by a single MN patch. This is the first report showing that MR vaccination delivered by MN patch can generate protective titers of neutralizing antibodies to both measles and rubella and provide complete protection from measles virus challenge in infant rhesus macaques.

The MN patch described in this report has the potential to overcome some of the logistical challenges associated with

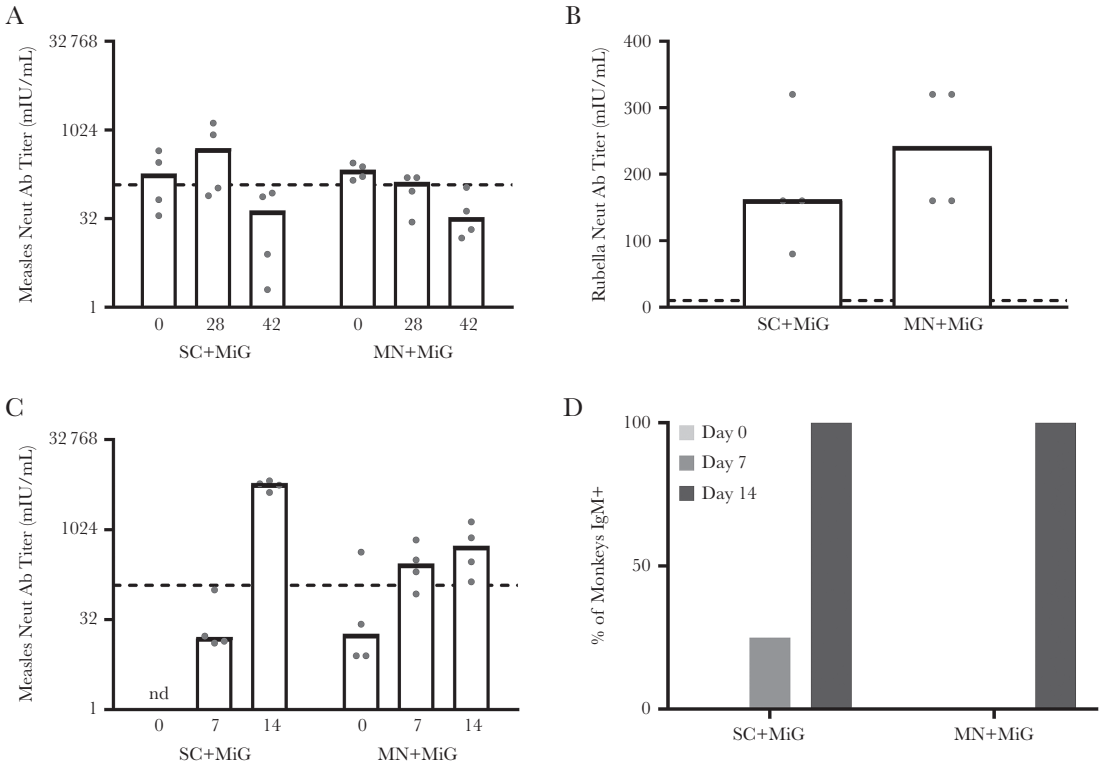

Figure 5. Neutralizing antibody (Neut Ab) titers to measles and rubella following vaccination of infant rhesus macaques with a microneedle (MN) patch or subcutaneous (SC) injection in the presence of measles immunoglobulin (MiG). *A* and *B*, Bars represent the median Neut Ab titer of each group; dots represent titers for each rhesus macaque. Measles-specific Neut Abs (*A*) were measured on days 0, 28, and 42. The dotted line indicates the minimum protective titer of 120 mIU/mL; none of the rhesus macaques had protective titers to measles on day 42. Rubella-specific Neut Abs (*B*) were measured on day 42. Dashed line indicates the protective titer of 10 IU/mL. The presence of MiG did not affect the response to rubella vaccine. *C* and *D*, Serologic response to measles following challenge with measles virus (see legend to Figure 4). All macaques in the MiG treatment group seroconverted to measles by day 14 (*C*), and all had an immunoglobulin M (IgM) response (*D*).

the delivery of MR vaccine by SC injection. MN patches have a single-dose presentation, which reduces vaccine wastage associated with use of multidose vials. Because the patches are administered directly to the skin, no vaccine reconstitution is needed, which eliminates errors associated with reconstitution. The needles dissolve after application to the skin so the used MN patches are not a sharps hazard, and the greatly reduced packing volume significantly reduces the amount of biological waste that must be disposed of after vaccination. Finally, minimally trained personnel can deliver MN patch vaccines, potentially allowing house-to-house mass vaccination, a strategy that has been extremely effective for the smallpox and polio eradication programs. We demonstrated that MN patches can be stored at elevated temperatures for at least 1 month without loss of activity, reducing cold chain requirements and permitting distribution of vaccines to remote locations; improved formulation that enables still better thermostability would be advantageous [10]. The cost of producing MN patch vaccines is expected to be similar to that of the injectable vaccines, and the reduction in cold chain requirements and associated vaccine wastage should make vaccination by MN patch cost-effective compared to current strategies [41].

MN patches have many advantages compared to other needle-free vaccination devices evaluated to deliver MR vaccine. Jet injectors utilize a stream of liquid at a high pressure to pierce the skin; however, administration of vaccine with jet injectors requires training, uses a bulky device, and offers no benefits for thermostability. In adults, delivery of measles, mumps, and rubella (MMR) vaccine with a jet injector induced an immune response comparable to SC injection but had significantly higher pain scores [42]. However, in another MMR vaccination study, the jet injector produced inferior antibody titers to MR compared with SC injection in a clinical trial in infants [43]. Measles vaccine was administered by the aerosol route via inhalation but gave inferior results compared to SC injection in a large phase 3 clinical trial [44]. Inhaled measles vaccine delivered as a dry powder vaccine gave promising results in nonhuman primates and a phase 1 clinical trial; however, the project was discontinued [45, 46].

As maternal antibodies begin to wane within 6 months of age, many infants become susceptible to measles before they are age-eligible for vaccination; this window of measles susceptibility accounts for approximately 20% of cases worldwide [47]. Novel measles vaccines, including subunit, live-vectored, and

DNA-based vaccines [39, 48–50], designed to generate protective immune responses in the presence of maternal antibody were tested in animal models, but none were evaluated in human clinical trials. Of these novel vaccines, the most promising results were reported for a DNA vaccine expressing the hemagglutinin, fusion, and N proteins of measles virus, which produced a protective response in the infant macaques in the presence of MiG. However, the DNA vaccine required coadministration with a molecular adjuvant, interleukin 2 [31, 36], which has not been approved for use in humans. Because MN patches provide efficient antigen presentation to the dendritic cells in the epidermal and dermal layers of the skin, which increased vaccine immunogenicity in other contexts [23–27], we tested the ability of intradermal delivery to overcome the inhibition of the immune response to measles vaccine caused by the presence of MiG. However, our findings indicated that the MN patch was unable to generate a protective immune response to measles in the presence of MiG.

Use of MN patches in public health settings will require developing manufacturing methods that enable cost-effective mass production (eg, $10e^6$–$10e^9$ patches/year), validating patch design to show that minimally trained personnel can reliably administer patches in diverse settings outside of healthcare locations, and conducting studies in humans to demonstrate safety and efficacy of MR vaccination by MN patch.

Overall, we conclude that MN patches offer a novel delivery method for MR vaccine that can eliminate many of the logistical challenges associated with currently available MR vaccines delivered by SC injection. Therefore, use of an MN patch could help increase MR vaccination coverage to the levels needed to achieve global and regional elimination goals. Considering the expected benefits of achieving MR elimination globally and the resulting reduction of MR morbidity and mortality, the development and licensure of the MN patch for MR vaccination has great potential value to global health.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The views expressed in this publication are those of the authors and do not necessarily represent the decisions, policy, or views of the CDC.

Financial support. J. C. J. was funded through the National Institute of General Medical Sciences of the National Institutes of Health sponsored Cell and Tissue Engineering Biotechnology Training Program (award number T32GM008433) and a National Science Foundation Graduate Research Fellowship (grant number DGE-1148903). Partial support was provided by the Global Immunization Division and National Center for Respiratory Diseases and Immunization of the CDC. Primate work at the University of California, Davis was supported by the Office of Research Infrastructure Programs of the NIH (award number P51OD011107).

Potential conflicts of interest. M. R. P. is an inventor of patents licensed to companies developing microneedle-based products; is a paid advisor to companies developing microneedle-based products; and is a founder/shareholder of companies developing microneedle-based products (Micron Biomedical). This potential conflict of interest has been disclosed and is managed by Georgia Tech and Emory University. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Rota PA, Moss WJ, Takeda M, de Swart RL, Thompson KM, Goodson JL. Measles. Nat Rev Dis Primers **2016**; 2:16049.
2. Global Vaccine Action Plan. Decade of vaccine collaboration. Vaccine **2013**; 31:B5–31.
3. Grant GB, Reef SE, Dabbagh A, Gacic-Dobo M, Strebel PM. Global progress toward rubella and congenital rubella syndrome control and elimination—2000–2014. MMWR Morb Mortal Wkly Rep **2015**; 64:1052–5.
4. Patel MK, Gacic-Dobo M, Strebel PM, et al. progress toward regional measles elimination—worldwide, 2000–2015. MMWR Morb Mortal Wkly Rep **2016**; 65:1228–33.
5. Arya J, Prausnitz MR. Microneedle patches for vaccination in developing countries. J Control Release **2016**; 240:135–41.
6. Prausnitz MR. Engineering microneedle patches for vaccination and drug delivery to skin. Annu Rev Chem Biomol Eng **2017**; 8:177–200.
7. Orenstein WA, Hinman A, Nkowane B, Olive JM, Reingold A. Measles and rubella global strategic plan 2012–2020 midterm review. Vaccine **2018**; 36:A1–34.
8. Cochi SL, Freeman A, Guirguis S, Jafari H, Aylward B. Global polio eradication initiative: lessons learned and legacy. J Infect Dis **2014**; 210:S540–6.
9. Henderson DA. The eradication of smallpox—an overview of the past, present, and future. Vaccine **2011**; 29:D7–9.
10. Marshall S, Sahm LJ, Moore AC. The success of microneedle-mediated vaccine delivery into skin. Hum Vaccin Immunother **2016**; 12:2975–83.
11. Quinn HL, Kearney MC, Courtenay AJ, McCrudden MT, Donnelly RF. The role of microneedles for drug and vaccine delivery. Expert Opin Drug Deliv **2014**; 11:1769–80.
12. Sullivan SP, Koutsonanos DG, Del Pilar Martin M, et al. Dissolving polymer microneedle patches for influenza vaccination. Nat Med **2010**; 16:915–20.

13. Kommareddy S, Baudner BC, Oh S, Kwon SY, Singh M, O'Hagan DT. Dissolvable microneedle patches for the delivery of cell-culture-derived influenza vaccine antigens. J Pharm Sci **2012**; 101:1021–7.
14. Demuth PC, Garcia-Beltran WF, Ai-Ling ML, Hammond PT, Irvine DJ. Composite dissolving microneedles for coordinated control of antigen and adjuvant delivery kinetics in transcutaneous vaccination. Adv Funct Mater **2013**; 23:161–72.
15. Edens C, Collins ML, Goodson JL, Rota PA, Prausnitz MR. A microneedle patch containing measles vaccine is immunogenic in non-human primates. Vaccine **2015**; 33:4712–8.
16. Mistilis MJ, Joyce JC, Esser ES, et al. Long-term stability of influenza vaccine in a dissolving microneedle patch. Drug Deliv Transl Res **2017**; 7:195–205.
17. Vrdoljak A, Allen EA, Ferrara F, Temperton NJ, Crean AM, Moore AC. Induction of broad immunity by thermostabilised vaccines incorporated in dissolvable microneedles using novel fabrication methods. J Control Release **2016**; 225:192–204.
18. Pearson FE, McNeilly CL, Crichton ML, et al. Dry-coated live viral vector vaccines delivered by nanopatch microprojections retain long-term thermostability and induce transgene-specific T cell responses in mice. PLoS One **2013**; 8:e67888.
19. Norman JJ, Arya JM, McClain MA, Frew PM, Meltzer MI, Prausnitz MR. Microneedle patches: usability and acceptability for self-vaccination against influenza. Vaccine **2014**; 32:1856–62.
20. Donnelly RF, Moffatt K, Alkilani AZ, et al. Hydrogel-forming microneedle arrays can be effectively inserted in skin by self-application: a pilot study centred on pharmacist intervention and a patient information leaflet. Pharm Res **2014**; 31:1989–99.
21. Rouphael NG, Paine M, Mosley R, et al. The safety, immunogenicity, and acceptability of inactivated influenza vaccine delivered by microneedle patch (TIV-MNP 2015): a randomised, partly blinded, placebo-controlled, phase 1 trial. Lancet **2017;** 390:649–58.
22. Edens C, Collins ML, Ayers J, Rota PA, Prausnitz MR. Measles vaccination using a microneedle patch. Vaccine **2013**; 31:3403–9.
23. Moon S, Wang Y, Edens C, Gentsch JR, Prausnitz MR, Jiang B. Dose sparing and enhanced immunogenicity of inactivated rotavirus vaccine administered by skin vaccination using a microneedle patch. Vaccine **2013**; 31:3396–402.
24. Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, Almagor Y, Sharon O, Levin Y. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. Vaccine **2009**; 27:454–9.
25. Koutsonanos DG, Esser ES, McMaster SR, et al. Enhanced immune responses by skin vaccination with influenza subunit vaccine in young hosts. Vaccine **2015**; 33:4675–82.
26. Puig-Barbera J, Natividad-Sancho A, Calabuig-Perez J, et al. Intradermal and virosomal influenza vaccines for preventing influenza hospitalization in the elderly during the 2011–2012 influenza season: a comparative effectiveness study using the Valencia health care information system. Vaccine **2014**; 32:5447–54.
27. Hung IFN, Levin Y, To KKW, et al. Dose sparing intradermal trivalent influenza (2010/2011) vaccination overcomes reduced immunogenicity of the 2009 H1N1 strain. Vaccine **2012**; 30:6427–35.
28. Esser ES, Romanyuk A, Vassilieva EV, et al. Tetanus vaccination with a dissolving microneedle patch confers protective immune responses in pregnancy. J Control Release **2016**; 236:47–56.
29. Chen MH, Zhu Z, Zhang Y, et al. An indirect immunocolorimetric assay to detect rubella virus infected cells. J Virol Methods **2007**; 146:414–8.
30. Hierholzer JC, Killington RA. Virus isolation and quantitation. In: Kangro HO, Mahy BW, eds. Virology methods manual. London: Academic Press, **1996**:25–46.
31. Premenko-Lanier M, Rota PA, Rhodes GH, Bellini WJ, McChesney MB. Protection against challenge with measles virus (MV) in infant macaques by an MV DNA vaccine administered in the presence of neutralizing antibody. J Infect Dis **2004**; 189:2064–71.
32. Zhu YD, Heath J, Collins J, et al. Experimental measles. II. Infection and immunity in the rhesus macaque. Virology **1997**; 233:85–92.
33. Hummel KB, Lowe L, Bellini WJ, Rota PA. Development of quantitative gene-specific real-time RT-PCR assays for the detection of measles virus in clinical specimens. J Virol Methods **2006**; 132:166–73.
34. Cohen BJ, Audet S, Andrews N, Beeler J; WHO Working Group on Measles Plaque Reduction Neutralization Test. Plaque reduction neutralization test for measles antibodies: description of a standardised laboratory method for use in immunogenicity studies of aerosol vaccination. Vaccine **2007**; 26:59–66.
35. Strebel P, Papania M, Dayan G, Halsey N. Measles vaccines. In: Plotkin S, Orenstein, W, Offit, P, eds. Vaccines. 5th ed. London: Saunders, **2004**:353–98.
36. Premenko-Lanier M, Hodge G, Rota P, Tamin A, Bellini W, McChesney M. Maternal antibody inhibits both cellular and humoral immunity in response to measles vaccination at birth. Virology **2006**; 350:429–32.
37. Premenko-Lanier M, Rota PA, Rhodes G, et al. DNA vaccination of infants in the presence of maternal antibody: a measles model in the primate. Virology **2003**; 307:67–75.
38. Niewiesk S. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. Front Immunol **2014**; 5:446.
39. Zhu YD, Rota P, Wyatt L, et al. Evaluation of recombinant vaccinia virus–measles vaccines in infant rhesus macaques

with preexisting measles antibody. Virology **2000**; 276:202–13.
40. Durrheim DN, Goodson JL. Time for an immunisation paradigm shift. Trans R Soc Trop Med Hyg **2017;** 111:41–2.
41. Adhikari BB, Goodson JL, Chu SY, Rota PA, Meltzer MI. Assessing the potential cost-effectiveness of microneedle patches in childhood measles vaccination programs: the case for further research and development. Drugs R D **2016**; 16:327–38.
42. Sarno MJ, Blase E, Galindo N, Ramirez R, Schirmer CL, Trujillo-Juarez DF. Clinical immunogenicity of measles, mumps and rubella vaccine delivered by the Injex jet injector: comparison with standard syringe injection. Pediatr Infect Dis J **2000**; 19:839–42.
43. de Menezes Martins R, Curran B, Maia Mde L, et al. Immunogenicity and safety of measles-mumps-rubella vaccine delivered by disposable-syringe jet injector in healthy Brazilian infants: a randomized non-inferiority study. Contemp Clin Trials **2015**; 41:1–8.
44. Low N, Bavdekar A, Jeyaseelan L, et al. A randomized, controlled trial of an aerosolized vaccine against measles. N Engl J Med **2015**; 372:1519–29.
45. Lin WH, Griffin DE, Rota PA, et al. Successful respiratory immunization with dry powder live-attenuated measles virus vaccine in rhesus macaques. Proc Natl Acad Sci U S A **2011**; 108:2987–92.
46. MVDP author group, Cape S, Chaudhari A, et al. Safety and immunogenicity of dry powder measles vaccine administered by inhalation: a randomized controlled phase I clinical trial. Vaccine **2014**; 32:6791–7.
47. Kumar ML, Johnson CE, Chui LW, Whitwell JK, Staehle B, Nalin D. Immune response to measles vaccine in 6-month-old infants of measles seronegative mothers. Vaccine **1998**; 16:2047–51.
48. Pasetti MF, Barry EM, Losonsky G, et al. Attenuated *Salmonella enterica* serovar Typhi and *Shigella flexneri* 2a strains mucosally deliver DNA vaccines encoding measles virus hemagglutinin, inducing specific immune responses and protection in cotton rats. J Virol **2003**; 77:5209–17.
49. Pasetti MF, Ramirez K, Resendiz-Albor A, Ulmer J, Barry EM, Levine MM. Sindbis virus-based measles DNA vaccines protect cotton rats against respiratory measles: relevance of antibodies, mucosal and systemic antibody-secreting cells, memory B cells, and Th1-type cytokines as correlates of immunity. J Virol **2009**; 83:2789–94.
50. Pasetti MF, Resendiz-Albor A, Ramirez K, et al. Heterologous prime-boost strategy to immunize very young infants against measles: pre-clinical studies in rhesus macaques. Clin Pharmacol Ther **2007**; 82:672–85.