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Dose sparing and enhanced immunogenicity of inactivated rotavirus vaccine administered by skin vaccination using a microneedle patch☆

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ABSTRACT

Skin immunization is effective against a number of infectious diseases, including smallpox and tuberculosis, but is difficult to administer. Here, we assessed the use of an easy-to-administer microneedle (MN) patch for skin vaccination using an inactivated rotavirus vaccine (IRV) in mice. Female inbred BALB/c mice in groups of six were immunized once in the skin using MN coated with 5 μg or 0.5 μg of inactivated rotavirus antigen or by intramuscular (IM) injection with 5 μg or 0.5 μg of the same antigen, bled at 0 and 10 days, and exsanguinated at 28 days. Rotavirus-specific IgG titers increased over time in sera of mice immunized with IRV using MN or IM injection. However, titers of IgG and neutralizing activity were generally higher in MN immunized mice than in IM immunized mice; the titers in mice that received 0.5 μg of antigen with MN were comparable or higher than those that received 5 μg of antigen IM, indicating dose sparing. None of the mice receiving negative-control, antigen-free MN had any IgG titers. In addition, MN immunization was at least as effective as IM administration in inducing a memory response of dendritic cells in the spleen. Our findings demonstrate that MN delivery can reduce the IRV dose needed to mount a robust immune response compared to IM injection and holds promise as a strategy for developing a safer and more effective rotavirus vaccine for use among children throughout the world.

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1. Introduction

Group A rotavirus is a major cause of severe diarrhea in children less than 5 years of age and is responsible for an estimated 453,000 deaths per year worldwide [1]. Two licensed live oral rotavirus vaccines, RotaTeq® and Rotarix™, have shown high efficacy among infants in developed and middle-income countries [2,3], but are less effective in low income countries of Africa, Asia, and Latin America where a vaccine is needed most [4–8]. Both vaccines are also associated with a small risk of gastroenteritis and intussusception among vaccinated infants [9,10]. These limitations are believed to be due largely to the use of live vaccines administered via the oral route [11]. To address these problems, we are developing an inactivated rotavirus vaccine (IRV) for parenteral immunization as an alternative approach to oral vaccination for infants throughout the world [12,13]. We previously showed that thermally inactivated rotavirus when formulated with aluminum hydroxide was highly immunogenic in mice [14]. We further demonstrated that intramuscular (IM) administration of our candidate IRV, i.e., CDC-9, induced high immunogenicity and protection against infection from oral challenge with a virulent human rotavirus strain in gnotobiotic piglets [15].

While IM injection of IRV has been successful, there are ongoing efforts to further increase the delivery of this parenteral vaccine. Here, we hypothesized that vaccination in the skin could improve the immunogenicity and possible dose sparing of IRV because skin has a special immunologic network [16,17]. The skin is extremely rich in antigen-presenting cells (APCs) that include Langerhans cells (LCs), dermal dendritic cells (DCs), macrophages and monocytes, as well as accessory cells such as keratinocytes [17–19]. These APCs recognize, uptake, and present foreign antigens to T and B cells in the draining lymph nodes to initiate adaptive immune responses. Cutaneous immunization has been effective in preventing infectious diseases, such as smallpox, tuberculosis and rabies [17,20] and has achieved dose sparing for a number of vaccines compared to IM or subcutaneous (SC) injection [21]. In addition, vaccination using hypodermic needles requires trained medical personnel and thus has limitations for mass vaccination. Furthermore, the generation of bio-hazardous sharp wastes and the concern from

☆ The finding and conclusions in this report are those of the authors and do not necessarily represent the views of CDC.

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needle-associated injuries and diseases are ongoing issues that could negatively impact vaccination coverage [22–24].

In this study, we investigated the use of microneedles (MNs) as a simple and reliable means to target IRV to the skin through the use of a patch that avoids the generation of hypodermic needle waste. MNs have been studied for the administration of a number of other vaccines and have shown evidence for dose sparing and increased immunogenicity compared to IM and SC delivery [25,26]. Using the tools of microfabrication, MN patches can be manufactured at low-cost for inexpensive mass production and can be administered painlessly, possibly by patients themselves.

2. Materials and methods

2.1. Preparation of IRV

CDC-9, a human G1P [8] rotavirus strain, was cultivated in Vero cells with roller bottle using Iscove's Modified Dulbecco's Medium (IMDM; Invitrogen, Grand Island, NY). Triple-layered particles (TLPs) were purified from culture supernatants by CsCl gradient centrifugation and inactivated by heat at 60 °C for 4 h [14]. Inactivated TLPs were concentrated by ultracentrifugation to a concentration of 3 mg/ml using the Bradford method in Hanks' balanced salt solution (HBSS) with $CaCl_2$ and $MgCl_2$ (Invitrogen) supplemented with 10% sorbitol (Sigma–Aldrich, St. Louis, MO) and stored at 4 °C before being coated onto MN arrays.

2.2. Fabrication of MN

MNs were fabricated from stainless steel sheets and coated with IRV using a procedure modified from a published study [27]. The MNs were 750 μm long and assembled in rows of 5 MN each. For coating, the vaccine at a concentration of 3 mg/ml in HBSS was diluted 1:1 with a coating buffer consisting of 15% w/v trehalose, 2% w/v carboxymethyl cellulose and 1% w/v Lutrol F68 (all from Sigma–Aldrich) dissolved in deionized water. This solution was used to increase the viscosity and the wettability of the vaccine solution, and the trehalose helped stabilize the vaccine during drying. The MNs were coated using an automated coating station developed at the Georgia Institute of Technology. Each MN was dipped into a well containing the vaccine in coating solution 7 times and then allowed to air-dry for approximately 5 min. The coated MNs were then placed in a sterile container which was sealed and stored overnight in a laminar flow hood. The seal was broken and the arrays were removed in the animal facility at the time of insertion. The needles were then packaged and stored at 25 °C overnight before use. To measure the amount of vaccine coated on each array of MNs, 6 arrays were put in a 0.5 ml tube and dissolved in 200 μl of phosphate-buffered saline (PBS) in triplicate. The protein concentration in the eluate was measured by Micro BCA Protein Assay kit (Pierce Biotechnology). The average amount coated on a five-needle MN array was 1 μg. Five of these arrays (i.e. a total of 25 MNs) were used to administer a 5 μg dose. To administer a 0.5 μg dose, the coating solution of 1.5 mg/ml IRV was used to prepare a single MN array with 0.5 μg IRV.

2.3. Immunization of mice

Female BALB/c mice (6–8 weeks old; Charles River Laboratories, Wilmington, MA) were used for the immunization study. All mice were anesthetized with 110 mg/kg ketamine (Ketathesia™, Butler Schein Animal Health, Dublin, OH) and 11 mg/kg xylazine (Anased®, Lloyd Laboratories, Shenandoah, IA) that were injected IM and were pre-bled from the submandibular vein with an animal lancet (Medipoint International, Mineola, NY). The hair on the back of the mice was shaved using electric sheers and the remaining hair was removed using a depilatory cream (Nair, Church & Dwight, Princeton, NJ). The area was then thoroughly cleaned using sterile water and alcohol swabs. After drying, mice in groups of six were immunized once by inserting IRV-coated MN (5 μg or 0.5 μg) on the back for 10 min or once by injecting IM 5 μg or 0.5 μg of IRV in 100 μl of PBS into the upper quadriceps muscles. For controls, mice in groups of six were immunized once with MN prepared using coating solution without IRV or once IM with 5 μg of IRV reconstituted from MN in 100 μl PBS. Mice were bled on day 10 and exsanguinated on day 28, when spleens were removed and placed in tubes containing RPMI 1640 media.

2.4. Measurement of cellular immune responses

Splenocytes were prepared as a cell suspension by gently pressing organ segments through a 70 μm nylon cell strainer (BD Falcon, Franklin Lakes, NJ) using a plastic pipette and then passed through the mesh. Spleen cell suspensions were depleted of red blood cells using Tris–NH_4Cl lysing buffer. The cells were washed twice with PBS plus 2% fetal bovine serum (Invitrogen, Grand Island, NY) and suspended in RPMI containing 20 mM HEPES, 100 U/ml of penicillin, and 100 mg/ml of streptomycin plus 10% fetal bovine serum. The cells were stored at −80 °C before use.

Splenocytes were thawed, counted and used for stimulation assay for dendritic cell responses by flow cytometry [28]. The cells (2×10^6 cells/ml) in IMDM were stimulated with either 200 μl of the supernatants of CDC-9 (7×10^7 fluorescence focus unit/ml)-infected Vero cells or 200 μl of the supernatants of mock-infected Vero cells (negative control). In addition, 10 μl of anti-CD28/anti-CD49d monoclonal antibody cocktail (BD Biosciences, San Diego, CA) was added to each sample as co-stimulator. Antigen stimulation was done in 15 ml polystyrene Falcon tubes (BD Falcon) by incubating at 37 °C with 5% CO_2 for 5 h, followed by another 5 h incubation with the addition of brefeldin A (10 μg/ml; Sigma–Aldrich), which blocked the secretion of cytokines from the cells. The cells were then washed once with 1× IMag™ buffer (BD Biosciences) and treated with 2 mM EDTA (Sigma–Aldrich) in PBS for 10 min to detach adherent cells. After washing with 1× IMag™ buffer, the cells were fixed with 1% paraformaldehyde (Sigma–Aldrich) for 5 min, washed, and then frozen at −70 °C in 1× IMag™ buffer with 10% dimethyl sulfoxide (Sigma–Aldrich) before use.

Frozen cells were thawed, washed once with 1× IMag™ buffer, and used for the purification of DCs using Dynabeads Mouse DC Enrichment Kit (Invitrogen Dynal AS, Oslo, Norway) according to the manufacturer's instructions. The DCs (93% pure) were incubated with 1× BD FACS permeabilizing soultion 2 (BD Bioscience) for 10 min, washed with 1× IMag™ buffer, and then stained for 30 min in the dark using a five-color assay with the following combinations of conjugated monoclonal antibodies: (1) MHCII-PE-Cy7/CD11c-Alex Fluor700/CD205-PE/CD11b-APC/CD80-FITC, (2) MHCII-PE-Cy7/CD11c-Alex Fluor700/CD205-PE/CD11b-APC/CD86-FITC, (3) MHCII-PE-Cy7/CD11c-Alex Fluor700/B220-PE/mPDCA1-APC/CD80-FITC, and (4) MHCII-PE-Cy7/CD11c-Alex Fluor700/B220-PE/mPDCA1-APC/CD86-FITC. Cells were also stained with PE-labeled, isotype-matched control monoclonal antibodies. PE-Cy7, Alexa Fluor 700, FITC, APC conjugates were obtained from BD Biosciences; anti-CD205-PE and anti-mPDCA1-APC conjugates were obtained from Mitenyi Biotec (Auburn, CA); and anti-MHCII-PE-Cy7 conjugate was obtained from eBioscience (San Diego, CA). All monoclonal antibodies were titrated and used at optimal concentrations. Stained cells were analyzed using a LSR II (BD Biosciences) and 30,000–50,000 events were counted. Data were analyzed with FACSDiva software (BD Biosciences) [29].