Use of Vaxfectin Adjuvant with DNA Vaccine Encoding the Measles Virus Hemagglutinin and Fusion Proteins Protects Juvenile and Infant Rhesus Macaques against Measles Virus

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A measles virus vaccine for infants under 6 months of age would help control measles. DNA vaccines hold promise, but none has provided full protection from challenge. Codon-optimized plasmid DNAs encoding the measles virus hemagglutinin and fusion glycoproteins were formulated with the cationic lipid-based adjuvant Vaxfectin. In mice, antibody and gamma interferon (IFN-γ) production were increased by two- to threefold. In macaques, juveniles vaccinated at 0 and 28 days with 500 μg of DNA intradermally or with 1 mg intramuscularly developed sustained neutralizing antibody and H- and F-specific IFN-γ responses. Infant monkeys developed sustained neutralizing antibody and T cells secreting IFN-γ and interleukin-4. Twelve to 15 months after vaccination, vaccinated monkeys were protected from an intratracheal challenge: viremia was undetectable by cocultivation and rashes did not appear, while two naïve monkeys developed viremia and rashes. The use of Vaxfectin-formulated DNA is a promising approach to the development of a measles vaccine for young infants.

Measles remains a major cause of infant mortality despite the availability of a safe and effective live attenuated virus vaccine. Recent efforts to reduce mortality through improved routine vaccination combined with mass vaccination campaigns have moved measles control toward the World Health Assembly goal of a 90% reduction in mortality by 2010 compared to the level of mortality in 2000 (5). One impediment to measles control remains the inability to immunize young infants due to the immaturity of the immune system and the interference of maternal antibodies that impair immune responses to the current vaccine (1, 11).

Because the waning of maternal antibodies varies from one infant to another, many children in areas with high levels of measles virus (MV) transmission are at risk of acquiring measles prior to vaccination (2, 3). This is particularly true of children born to human immunodeficiency virus-positive mothers who have lower levels of maternal antibodies at birth (26, 40). Independently of maternal antibodies, immaturity affects the quality and quantity of antibody produced in response to the current live attenuated vaccine, with lower levels of neutralizing antibody and deficient avidity and isotype maturation compared to those of older infants (11, 28, 42). As a result, the recommended age for vaccination generally is 9 months in developing countries and 12 months in developed countries, which balances the risk of infection with the likelihood of a response to the vaccine (14).

A vaccine that could be given under the age of 6 months would improve measles control by allowing delivery with other infant vaccines and by closing the window of susceptibility prior to the delivery of the current vaccine. Increasing the dose of vaccine improved the antibody responses in young infants but resulted in an unexpected increase in mortality for girls, so this is not a viable approach to lowering the age of vaccination (12, 17). Therefore, other strategies are necessary for the development of a vaccine for young infants.

MV encodes six structural proteins, of which two, hemagglutinin (H) and fusion (F), are surface glycoproteins involved in attachment and entry. Antibodies that inhibit MV infection in neutralization assays are directed primarily against the H protein, which also contains important CD8+ T-cell epitopes (30), with some contribution from the F protein (36). Because protection from measles correlates best with the quality and quantity of neutralizing antibodies at the time of exposure (6, 36), most experimental vaccines have used H alone or H and F for the induction of MV protective immunity (32, 36, 46, 50).

Several small-animal models are available for testing new measles vaccines, but only nonhuman primates develop disease after infection with wild-type strains of MV so that protective immunity and vaccine safety can be assessed. Experimental vaccines that have been tested in nonhuman primates include iscoms (immunostimulatory complexes) (8, 44), recombinant viral vectors (32, 45, 46, 50), recombinant bacterial vectors (49), and DNA (34, 36, 43). DNA vaccines are attractive candidates for development, because they do not elicit antivector immunity, are safe, are relatively inexpensive to produce, may

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not require a cold chain, and induce strong cellular immune responses (39). However, DNA vaccines often have been disappointing when tested in humans and nonhuman primates because of the relatively poor induction of antibody (9). Unformulated DNA vaccines encoding MV H, F, or H and F (designated H+F) proteins induce sustained antibody responses of various titers and provide partial protection from challenge in juvenile rhesus monkeys (36, 37), but infant monkeys have poor responses (F. P. Polack, S. Lydi, P. Rota, S. H. Lee, S. J. Hoffman, R. J. Adams, H. L. Robinson, and D. E. Griffin, unpublished data), suggesting that the vaccine needs improvement. Approaches to improving responses to DNA vaccines have included increasing the amount of DNA given, using a microparticle formulation, improving the plasmids, altering the delivery, and adding adjuvants (7, 19–21).

One class of adjuvants is based on cationic lipid systems. Cationic lipid systems can be easily manufactured and are safe and well tolerated in humans and other animals (27, 33). Vaxfectin is a recently introduced cationic lipid-based adjuvant for DNA vaccines that consists of an equimolar mixture of the cationic lipid GAP-DOMORIE [(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(cis-9-tetradecenoyloxy)-1-propanaminobromide] and a neutral colipid, DPyPE (1,2-diphtanoylsn-glycero-3-phosphoethanolamine) (15). Vaxfectin is dose sparing, enhances the production of antigen-specific antibody in small animals, including virus-neutralizing antibody, and can induce immunity to a variety of infections (13, 15, 16, 18, 24, 29, 41). However, the efficacy of Vaxfectin-formulated DNA vaccines has not been reported for humans, and there is only a single study of nonhuman primates (22). No studies have examined its efficacy in very young animals.

In the current study, we evaluated Vaxfectin-formulated plasmid DNAs expressing codon-optimized H and F proteins as a potential MV vaccine. Vaxfectin improved antibody and T-cell responses to MV in mice. The Vaxfectin-formulated DNA vaccine induced a sustained production of neutralizing antibodies in both juvenile and infant monkeys after two intramuscular (i.m.) or intradermal (i.d.) injections. More than a year after vaccination, all monkeys were completely protected against rash and viremia when challenged with wild-type MV.

MATERIALS AND METHODS

Animals. Six-week-old female BALB/c mice were purchased from Charles River Breeding Laboratories (Wilmington, MA). Twelve 2-year-old juvenile and four 1-month-old infant rhesus macaques (Macaca mulatta) born to measles-naive mothers were obtained from the Johns Hopkins Primate Breeding Facility. Monkeys were chemically restrained with ketamine (10 to 15 mg/kg of body weight) during procedures. All animals were maintained within the guidelines of, and studies were performed in accordance with, experimental protocols approved by the Animal Care and Use Committee for Johns Hopkins University.

Vaccine. Coding nucleotide sequences for the H and F antigens of the Moraten strain of MV were codon optimized for expression in humans. Resulting DNA sequences were synthesized by GeneArt (Regensburg, Germany) and subcloned into expression plasmid VR1012 to create VR-H and VR-F. Coding nucleotide sequences for the H and F antigens of the Edmonston strain of MV were cloned into expression plasmid pGAT (from J. Peranen, Institute of Biotechnology, University of Helsinki, Finland) and into VR1012. Expression from VR-H, pGAT-H, VR-F, and pGAT-F was confirmed by the transient transfection of mouse VERO cells, followed by Western blot analysis of VR-H and VR-F plasmids. VR-H and VR-F plasmids were formulated with Vaxfectin as first described by Hartikka and coworkers (15). Briefly, both GAP-DOMORIE and DPyPE were resuspended in chloroform, mixed in 1:1 molar ratios, aliquoted into vials, and dried to create Vaxfectin reagent dry lipid film. On the day of injection, the lipid film vials were resuspended in 1 ml 0.9% saline and diluted, if necessary. Plasmid DNA was prepared in 0.9% saline, 20 mM sodium phosphate, pH 7.2. Plasmid DNA was formulated with Vaxfectin by gently streaming the lipid into plasmid DNA of equal volume. All of the required doses were prepared by being formulated at 0.2 to 0.5 mg/ml and diluted down to a lower concentration as required. The final DNA/cationic lipid molar ratio was 4:1.

Immunization of mice. Groups of 6 mice received 1, 3, 10, 30, or 100 μg of Vaxfectin-formulated VR-H and VR-F with the codon-optimized Moraten sequences, 30 μg VR-H and VR-F with the Edmonston sequences, 15 μg VR-H, 15 μg VR-F, 100 μg empty VR1012 plasmid or 100 μg pGAT-H, and pGAT-F without Vaxfectin i.m. A second dose was delivered 4 weeks later. Mice were bled at 2-week intervals for the measurement of antibody levels, and spleens were collected at 4 weeks for the assessment of the cellular immune response.

Vaccination and challenge of monkeys. Five juvenile rhesus macaques were immunized with Vaxfectin-formulated VR-H (500 μg) and VR-F (500 μg) i.m. Five juvenile and four infant rhesus macaques were immunized with Vaxfectin-formulated VR-H (250 μg) and VR-F (250 μg) i.d. (five sites for each plasmid). All monkeys were boosted 4 weeks later. One infant monkey died 10 weeks after immunization of unrelated causes. Juvenile monkeys were bled at 2- to 4-week intervals, and infant monkeys were bled at monthly intervals after vaccination. Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by Ficoll-Paque (Amersham Pharmacia) gradient centrifugation. Plasma was collected and stored at −20°C.

All monkeys were challenged intratracheally with 103 to 106 50% tissue culture infective doses (TCID50) of the wild-type Bithlostrain strain of MV (A. Osterhaus, Erasmus University, Rotterdam, The Netherlands). Juvenile monkeys were challenged 15 months and infant macaques 12 months after the first vaccination, along with two naïve juvenile monkeys. All monkeys were bled at regular intervals to monitor viremia and immune responses after challenge.

Virus assays. Viremia was assessed by the cocultivation in triplicate of serial dilutions (103 to 105) of PBMCs with B95-8 maromos B cells in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin. Wells were scored at 96 h for MV-positive syncytia. Data are reported as numbers of syncytia/106 PBMC.

ELISPOT assays. Neutralizing antibodies were measured by the ability of serially diluted plasma to reduce plaque formation by the Chicago-1 strain of MV on Vero cells (i.e., plaque reduction neutralization [PRN]). The international standard serum 66/202 was included in all assays, and data were normalized to that standard to calculate international units (IU) of neutralizing antibody per milliliter.

For enzyme immunoassays (EIAs), MV-infected Vero cell lysate (Advanced Biotechnologies, Columbia, MD) was used (1.16 μg protein/well) to coat 96-well Maxisorp plates (Nunc, Rochester, NY) and then incubated with serially diluted plasma overnight at 4°C. For mice, a horseradish peroxidase (HRP)-conjugated sheep antibody to mouse immunoglobulin G (IgG) (Amersham) was the secondary antibody, and TMB (R&D Systems) was the substrate. A laboratory standard serum was included in each plate, and data are presented as ELA units (EU) per μl of plasma. Titers of antibodies were diluted 1:400 for mice and 1:200 (IgM), and an alkaline phosphatase-conjugated rabbit antibody to monkey IgG (Biomakor; Accurate Chemicals, Westbury, NY) or HRP-conjugated goat antibody to monkey IgM (Nordic, Capanistrano Beach, CA) was used as the secondary antibody. Data are presented as optical density (OD) values.

To measure the avidity of MV-specific IgG, 50 μl of increasing concentrations (0 to 3.5 M) of ammonium thiocyanate (NH4SCN) were added to EIA plates after incubation with plasma (1:100). Plates were washed, and the rabbit anti-monkey IgG was added as described above. The avidity index was calculated as the concentration of NH4SCN at which 50% of the bound antibody was eluted (28).

ELISPOT assays. For mice, spleen cells were harvested, incubated with red blood cell lysis buffer (Sigma), washed, and suspended in RPMI supplemented with 10% FBS, 2 mM l-glutamine, penicillin, and streptomycin. Multiscreen plates (Millipore) were coated with antibody to mouse gamma interferon (IFN-γ) or interleukin-4 (IL-4) (5 μg/ml; BD Pharmingen, San Diego, CA). Plates were washed and blocked with culture medium, and 1 × 105 to 5 × 106 splenocytes were added along with 1 μg/ml pooled MV H or F peptides (20-mer overlapping by 11 amino acids) (30, 32), 5 μg/ml of concanavalin A (ConA; Sigma, St. Louis, MO), or medium. After 4 h of incubation, plates were washed and incubated with 2 μg/ml biotinylated antibody to mouse IFN-γ or IL-4 for 2 h at 37°C. For monkeys, 1 × 105 to 5 × 106 PBMCs were added to plates coated with antibody to human IFN-γ (2 μg/ml) or IL-4 (5 μg/ml) (BD Pharmingen) along with 1 μg/ml pooled MV H or F peptide, 5 μg/ml of ConA, or medium. After 4 h at 37°C, plates were washed and incubated with 1 μg/ml biotinylated antibody to IFN-γ (Milabtech) or 2 μg/ml...
biotinylated antibody to IL-4 (PharMingen) at room temperature for 2 h. After washing, 50 μl H9262 of HRP-conjugated avidin (Research Laboratory Inc.) was added to each well and incubated for 1 h at 37°C. The assays were developed with 50 μl stable diaminobenzidine solution (Invitrogen, Carlsbad, CA) for 10 min. Wells were scanned in an ImmunoSpot reader and analyzed using ImmunoSpot 2.0.5 software (C.T.L., Cleveland, OH). Data are presented as the number of spot-forming cells (SFCs) per 10^6 splenocytes or 10^6 PBMCs after the subtraction of the medium control.

**Statistical analysis.** Student’s unpaired t test or one-way analysis of variance was used for the comparison of responses between groups of monkeys by using Prism 4 software.

**Nucleotide sequence accession numbers.** Nucleotide sequences for the encoding of codon-optimized H and F antigens were deposited in GenBank under accession numbers EU723762 and EU723763, respectively.

### RESULTS

**Immune responses in mice.** To determine whether the codon optimization of the DNA H and F sequences or Vaxfectin adjuvant improves the immunogenicity of an MV DNA vaccine, mice were immunized with nonoptimized or codon-optimized H and F formulated either with (VR-H and/or VR-F) or without (pGAT) Vaxfectin (Fig. 1). MV-specific IgG was induced in all MV-immunized groups, reached a peak soon after the boost at 4 weeks, and was sustained at a high level through 26 weeks (Fig. 1A). The peak IgG titer was higher for 100 μg VR-H+F (4,646 ± 413 EU/ml) than for 100 μg pGAT-H+F (1,660 ± 392 EU/ml; P < 0.05) and was higher for 30 μg codon-optimized VR-H+F (3,182 ± 807) than for 30 μg nonoptimized VR-H+F (1,269 ± 164). The antibody response to Vaxfectin-formulated DNA was mostly dose dependent for both EIA (Fig. 1B) and PRN (Fig. 1C). VR-H (15 μg) elicited higher IgG and neutralizing antibody responses than 15 μg VR-F or 30 μg VR-H+F (Fig. 1B and C).

Spleen cell H- and F-specific IFN-γ responses were assessed by ELISPOT assay. The highest response was in the group receiving 30 μg VR-H+F (H, 327 ± 18; F, 347 ± 31) (Fig. 1D), which also was higher than the response to 30 μg nonoptimized VR-H+F (H, 112 ± 5; F, 75 ± 19). MV-specific IL-4 production was not detected (data not shown). Based on these results with mice, subsequent studies of rhesus macaques used Vaxfectin-formulated, codon-optimized VR-H+F for vaccination.

**Immune responses in rhesus macaques.** To evaluate the route of administration, immunogenicity, and protection from
measles in nonhuman primates, groups of five juvenile rhesus macaques were immunized with 500 μg VR-H+F i.m. or 1 mg VR-H+F i.d. Four 1-month-old infant monkeys received 500 μg VR-H+F i.d. Four weeks later, all monkeys were boosted with the same doses by the same routes. After the first dose, all juvenile and three of four infant monkeys had levels of MV-specific neutralizing antibodies above the generally recognized protective level (120 mIU/ml) (6) (Fig. 2A). The maximum PRN antibody levels were achieved in juvenile macaques 2 weeks after the 4-week boost and were sustained above the protective level for more than 1 year. Infant macaques could be assessed less frequently, but they showed a similar pattern. The geometric mean peaks of neutralizing antibody for juvenile monkeys were 8,710 ± 2,123 mIU/ml after i.m. administration and 7,943 ± 1,425 mIU/ml after i.d. administration. For infant monkeys, the mean peak was 3,561 ± 1,400 mIU/ml. There were no significant differences in the peak responses between i.m. and i.d. groups or juvenile and infant monkeys, but at week 20 infant responses were lower than those of either i.m.- or i.d.-vaccinated juvenile monkeys (P < 0.05). MV-specific IgG EIA responses were induced in all VR-H+F-immunized monkeys, with a time course similar to that of the development of neutralizing antibody (Fig. 2B).

PBMC H-specific (Fig. 2C) and F-specific (Fig. 2D) T-cell responses were assessed using IFN-γ and IL-4 ELISPOT assays. All juvenile monkeys developed high IFN-γ and low IL-4 production (Fig. 2E). IFN-γ responses showed a peak in SFCs 2 weeks after vaccination, a slight increase after the boost, and were detectable for more than a year. Responses to H were higher than those to F in all juvenile monkeys. Peak H-specific SFCs/10^6 PBMC were 95 ± 23 for i.m. and 112 ± 17 for i.d. groups, while F-specific SFCs/10^6 PBMC were 32 ± 13 for i.m. (P = 0.044) and 52 ± 13 for i.d. (P = 0.035) groups. For infant monkeys, the IFN-γ responses to H (15 ± 7) were lower than those in the juvenile monkeys (P = 0.029), with similar responses to F (17 ± 12). These young monkeys developed IL-4 SFCs (H, 22 ± 9; F, 15 ± 10) that were comparable to the IFN-γ SFC response (Fig. 2E).

Protection of immunized monkeys from wild-type MV challenge. Twelve to 15 months after immunization, all vaccinated monkeys, plus two naïve monkeys, were challenged with wild-type MV. At the time of challenge, neutralizing antibody levels for all vaccinated juvenile monkeys (geometric mean, 589 ± 113 mIU/ml for the i.m. group; 527 ± 105 mIU/ml for the i.d. group) and two of three infant monkeys (610, 203, and 57 mIU/ml) were predicted to be protective. Between 9 and 11 days after challenge, both naïve animals developed rashes on the face and trunk, while none of the vaccinated monkeys developed rashes. Naïve monkeys developed viremias with a mean peak of 10^2.5 TCID_{50}/10^6 PBMC, while none of the

FIG. 2. Immune responses of rhesus macaques to Vaxfectin-formulated DNAs expressing H and F. Groups of five juvenile monkeys or four infant monkeys were immunized with 1 mg of VR-H+F i.m. or 500 μg of VR-H+F i.d. and were boosted 4 weeks later (arrow). One infant monkey died of unrelated causes 10 weeks after immunization. (A) MV-specific neutralizing antibodies were measured by plaque reduction. The protective level of neutralizing antibodies is shown with a solid line. Data are presented as the geometric means (in mIU per milliliter) ± SEM. (B) MV-specific IgG was measured by EIA. Data are expressed as ODs ± SEM for plasma diluted 1:400. (C) H-specific and (D) F-specific T-cell responses to pooled peptides were measured by IFN-γ ELISPOT assays. (E) Peak H-specific IFN-γ and IL-4 T-cell responses. Data are presented as the mean number of SFCs per million PBMCs ± SEM. * P < 0.05 by analysis of variance.
vaccinated juvenile or infant monkeys developed viremia that was detectable by cocultivation (Fig. 3A).

Antibody responses after challenge. Naive monkeys showed a high MV-specific IgM response, with peak ODs (0.71 ± 0.02) at day 15, while juvenile monkeys immunized either i.m. or i.d. showed no change in IgM from the baseline level (OD = 0.18 ± 0.02) (Fig. 3B). Previously vaccinated infant monkeys had a transient IgM increase at day 10 (OD = 0.4 ± 0.06).

Neutralizing antibody responses in unvaccinated control animals appeared 10 days after challenge and continued to increase for months, while titers increased only slightly in juvenile monkeys vaccinated either i.m. or i.d. (Fig. 4A). The levels of neutralizing antibodies increased 10-fold in infant monkeys. All vaccinated monkeys had detectable MV-specific IgG before challenge, with a mean OD of 0.377 ± 0.05 for the juvenile i.m. group, 0.316 ± 0.03 for the juvenile i.d. group, and 0.25 ± 0.07 for the infant i.d. group (Fig. 4B). After challenge, IgG levels increased minimally (0.492 ± 0.09 at day 20) for juvenile monkeys immunized i.m., while they increased to 0.835 ± 0.21 (day 20) in the i.d. group and to 1.057 ± 0.15 (day 18) for infant monkeys.

All vaccinated monkeys showed a high avidity index for MV-specific IgG before challenge, with a mean of 1.5 ± 0.14 for juvenile monkeys immunized i.m., 1.5 ± 0.03 for juvenile monkeys immunized i.d., and 1.6 ± 0.24 for infant monkeys immunized i.d. (Fig. 4C). After challenge, IgG avidity increased in all vaccinated monkeys and reached a peak 18 to 20 days after challenge, and then the levels decreased and reached a plateau that was greater than the prechallenge values (2.2 ± 0.14 for the i.m. group, 1.9 ± 0.1 for the i.d. group, and 2.0 ± 0.04 for infants). The unvaccinated control monkeys showed a slow rise in avidity that was 1.2 ± 0.2 at day 50.

Cellular immune responses after challenge. ELISPOT assays of the PBMC production of IFN-γ were used to monitor the H- and F-specific T-cell responses to virus challenge. All vaccinated monkeys showed a rapid rise in the production of IFN-γ in response to H or F peptide stimulation that peaked at days 14 to 20 after challenge and then retracted to a stable level above the prechallenge baseline. Infant monkeys had the highest level of IFN-γ production. The development of MV-specific IFN-γ-producing cells was slower for unvaccinated control monkeys, with a peak at day 29, indicating an anamnestic response in immunized monkeys (Fig. 5A and B). The peak H-specific IFN-γ SFC number was 33 ± 6 for i.m., 57 ±
Immunization with Vaxfectin-formulated, codon-optimized DNAs expressing the MV H and F proteins elicited strong antibody and T-cell responses in mice and rhesus macaques and provided protection against both rash and viremia after challenge with wild-type MV in infant and juvenile macaques. Two doses of vaccine delivered either i.d. or i.m. to juvenile or i.d. to infant rhesus macaques induced MV-specific antibody responses that were durable, neutralizing, and of high avidity, as well as inducing MV-specific IFN-γ-producing memory T cells. This is the first DNA-based MV vaccine that has successfully immunized infant macaques and the first to provide long-term protection from measles rash and viremia for both infant and juvenile macaques. A Vaxfectin-formulated MV DNA vaccine represents a candidate for further development as a new measles vaccine for young infants.

In mice, a variety of experimental DNA vaccines formulated with Vaxfectin have increased antibody production up to 100-fold above that of naked DNA, particularly at low doses, and produced a more durable response (13, 24, 29, 38). In the current study, these advantages were confirmed for DNA expressing MV H and F. Similar improvements also have been observed in studies of immune responses in rabbits and sheep (15, 16, 41). Improvement varies with the antigen (38), and in the current study antibody responses against the MV H protein were 2 to 10 times higher for DNA formulated with Vaxfectin than with phosphate-buffered saline. Responses were dose dependent and had not reached a plateau at 100 μg of DNA. H induced 10 times higher IgG titers than F at the same dose, and this may reflect differences in the immunogenicity of the proteins or in the levels of protein expression. The mechanisms by which Vaxfectin improves antibody responses are not fully understood. It does not improve the transfection of myocytes after i.m. inoculation, but it may improve the transfection of other cells or delivery to antigen-presenting cells (15, 47).

Vaxfectin is immunostimulatory and may act through the increased production of IL-6 and IFN-γ, cytokines that activate B cells and facilitate differentiation to plasma cells (15, 38).

T-cell responses to DNA vaccines in mice also are improved by Vaxfectin formulation (13, 18). Previous studies of the MV DNA immunization of mice have shown improved cytotoxic T-cell responses to MV H by the use of cationic lipids for mucosal vaccines (10). In the current study, T-cell responses were characterized by high IFN-γ and low IL-4 production, consistent with other reports that Vaxfectin preserves the Th1 cytokine profile typical of DNA vaccines (29, 38). Interestingly, the induction of IFN-γ-producing SFCs was less dose dependent than the antibody response and was more balanced with respect to H and F. This may reflect different requirements for the induction of antibody and T-cell responses to these proteins.

The induction of protective immune responses in nonhuman primates by MV DNA vaccines has been challenging. Previous studies of an unformulated H+F MV DNA vaccine delivered i.d. or by gene gun to juvenile macaques showed good T-cell responses, antibody responses that were sustained, and protection in monkeys with PRN antibody values of >200 mIU/ml at the time of challenge, but not all monkeys achieved this level (36). The formulation of an alphavirus-based DNA vaccine with polylactide coglycolide microspheres did not improve immune responses, and low doses delivered i.d. primed for enhanced disease after challenge (31). Formulation with Vaxfectin substantially improved the predictability, magnitude, and kinetics of the antibody and T-cell responses in juvenile macaques. Within 1 month, all juvenile monkeys immunized either i.d. or i.m. developed protective levels of neutralizing antibody that were similar to those previously reported for rhesus macaques after immunization with the current live measles vaccine (with an average of 4,943 mIU/ml) (36). In prior studies of the responses of juvenile monkeys to similar doses of unformulated, non-codon-optimized H+F DNA delivered i.d., mean PRN antibody levels were 85 mIU/ml after a single dose and 382 mIU/ml after a second dose (36). The MV-specific IFN-γ response also was rapid, with a peak 2 weeks after vaccination. The need for the second dose at 4 weeks is un-
clear, as the antibody response still was rising at the time of the boost and the T-cell responses increased little after the boost. The only previous report of the efficacy of a Vaxfectin-formulated DNA vaccine in nonhuman primates showed the induction of T-cell responses and the partial protection of baboons from challenge with human immunodeficiency virus type 2 (22).

The immaturity of the immune system is a barrier to early immunization for measles as well as other infectious diseases (4). Previous studies of infant monkeys have shown the priming of the immune response by naked DNA but limited protection from challenge unless boosted with the live virus vaccine (34, 43). Studies of neonatal immunization have been performed in mice. Some studies have suggested that DNA vaccines are tolerizing (25), but most have demonstrated good antibody and T-cell responses even in the face of maternal antibody (4, 23, 48). In general, the T-cell responses in young animals tend to be more skewed toward Th2 cytokines than the responses of older animals (23). In a previous report, the immunization of 1- to 2-week-old macaques with two doses of vaccinia virus-vectored MV H+F induced various levels of neutralizing antibodies, cytotoxic T-cell responses, and protection from challenge 12 weeks after boosting (50). In the current study, antibody responses were similar to those for juvenile monkeys, but T-cell responses were not. Juvenile monkeys developed predominantly IFN-γ-producing T cells with better responses to H than F. The IFN-γ responses of infant monkeys were lower than those of juvenile monkeys, and the numbers of IFN-γ- and IL-4–producing T cells were similar, as were the responses to H and F.

Both infant and juvenile monkeys developed sustained neutralizing antibody titers that were higher than 120 mIU/ml and were protected from measles, as determined by the development of rash and the detection of viremia. The antibody was of high avidity, a property previously shown to be important for the neutralization of wild-type virus and the prevention of exacerbated disease after challenge (35). However, infant monkeys did develop a transient IgM response and a 10-fold increase in neutralizing antibodies after challenge, indicating some virus replication, at least locally in the lung and draining lymph nodes. Although juvenile monkeys showed no increase in IgM, neutralizing antibody, or EIA antibodies, there was an increase in the avidity of IgG after challenge, which also was observed after measles infection in monkeys previously vaccinated with the current live virus vaccine (35), again indicating the stimulatory effects on the immunologic memory of limited virus replication upon reexposure. Infant monkeys also showed an increase in IFN-γ, but not IL-4, responses after challenge, potentially reflecting the maturation of the immune system during the after vaccination.

These studies did not address the second challenge to infant vaccination, the ability to induce a protective immune response in the presence of maternal antibody. There should be no impediment to the expression of MV proteins by DNA, as there is with the live virus vaccine when replication is inhibited by the presence of maternal antibody. However, immune responses can be suppressed by antibodies, and the immunogenicity of a Vaxfectin-formulated H+F vaccine will need to be directly tested in young infants in the presence of antibody. Likewise, it will be of interest to assess the responses of vaccinated monkeys to the current live attenuated virus vaccine, as the most likely use for any vaccine given under the age of 6 months will be to provide priming for and protection prior to immunization with the current vaccine.

In summary, our data provide the first candidate measles DNA vaccine that can elicit rapid and sustained protective responses to measles in infant monkeys as well as juvenile monkeys. A Vaxfectin-formulated DNA vaccine is a promising approach for the development of a new measles vaccine for young children.

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