Evaluation of Recombinant Vaccinia Virus—Measles Vaccines in Infant Rhesus Macaques with Preexisting Measles Antibody

Yong-de Zhu,*1 Paul Rota,† Linda Wyatt,‡ Azaibi Tamin,† Shmuel Rozenblatt,‡ Nicholas Lerche,* Bernard Moss,‡ William Bellini,† and Michael McChesney*§3

*The California Regional Primate Research Center and §Department of Pathology, School of Medicine, University of California, Davis, California 95616; †Measles Virus Section, Respiratory and Enteric Viruses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333; and ¶Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Received June 20, 2000; returned to author for revision July 28, 2000; accepted August 1, 2000

Immunization of newborn infants with standard measles vaccines is not effective because of the presence of maternal antibody. In this study, newborn rhesus macaques were immunized with recombinant vaccinia viruses expressing measles virus hemagglutinin (H) and fusion (F) proteins, using the replication-competent WR strain of vaccinia virus or the replication-defective MVA strain. The infants were boosted at 2 months and then challenged intranasally with measles virus at 5 months of age. Some of the newborn monkeys received measles immune globulin (MIG) prior to the first immunization, and these infants were compared to additional infants that had maternal measles-neutralizing antibody. In the absence of measles antibody, vaccination with either vector induced neutralizing antibody, cytotoxic T cell (CTL) responses to measles virus and protection from systemic measles infection and skin rash. The infants vaccinated with the MVA vector developed lower measles-neutralizing antibody titers than those vaccinated with the WR vector, and they sustained a transient measles viremia upon challenge. Either maternal antibody or passively transferred MIG blocked the humoral response to vaccination with both WR and MVA, and the frequency of positive CTL responses was reduced. Despite this inhibition of vaccine-induced immunity, there was a reduction in peak viral loads and skin rash after measles virus challenge in many of the infants with preexisting measles antibody. Therefore, vaccination using recombinant vectors such as poxviruses may be able to prevent the severe disease that often accompanies measles in infants.

INTRODUCTION

Measles virus (MV) is a negative-strand RNA virus in the Morbillivirus genus of the family Paramyxoviridae, and it is the causative agent of measles (Griffin and Bellini, 1996). Measles is a highly contagious childhood disease characterized by fever and respiratory and systemic infection. Measles in the infant is frequently associated with serious complications and a high mortality rate. Measles cases have dropped dramatically worldwide since the introduction of live, attenuated vaccines in the 1960s, especially in the developed countries. There were only 100 reported cases in the U.S. in 1999 (CDC, 2000), and the interruption of indigenous MV transmission in the U.S. has been maintained since late 1993 (CDC, 1996; Watson et al., 1998). Although the currently used live, attenuated measles vaccines are very safe and effective, they are neutralized in vivo by maternal antibody (Albrecht et al., 1977). To avoid the interference of maternal antibody, vaccine is administered at the age of 9 months in developing countries and at 12–15 months of age in the developed nations. The existence of a window of susceptibility to infection, between the time of decay of maternal antibody below a protective level and the time of vaccination, contributes to a worldwide childhood measles mortality of 1,000,000 annually, mainly in the developing countries (World Health Organization, 1996). Recently, several reports demonstrated that infants even less than 4 months old can contract measles (Oshitani et al., 1998; Wairagkar et al., 1998). The use of high-titered or inactivated measles vaccines was found to be unacceptable as a means to overcome the interference of maternal antibody (Aaby et al., 1988, 1993; Fulghini et al., 1967; Halsey, 1993; Kumar et al., 1998; Whittle et al., 1988).

The two envelope glycoproteins of MV, the hemagglutinin (H) and fusion (F) proteins, are important components of a measles vaccine. During MV infection, the H and F proteins elicit both neutralizing antibodies and cellular immunity. Neutralizing antibodies prevent the spread of extracellular virus and the cellular immune response plays a critical role in the recovery from infec-
tion (Burnet, 1968; Good and Zak, 1956). CTL epitopes have been mapped to H and F in a human population (Jaye et al., 1998a,b). Thus, the expression of MV H and F in a recombinant vector like vaccinia virus might be able to circumvent the effect of maternal antibody and to immunize the newborn host to H and F antigens.

Unlike rodents, Asian macaques contract measles naturally or experimentally, and the clinical signs of measles are similar to those in humans; thus, the monkey is a practical model for the study of measles pathogenesis and for the development of new measles vaccines (Griffin and Bellini, 1996; Hall et al., 1971; Kobune et al., 1996; McChesney et al., 1997; van Binnendijk et al., 1994). Several studies have shown that recombinant vaccinia viruses expressing the MV H and F proteins can protect against MV infection in both mice and monkeys (Drillien et al., 1988; van Binnendijk et al., 1997; Wild et al., 1992). The highly attenuated modified vaccinia Ankara (MVA) has been used as a recombinant expression vector and is being developed as candidate vaccine for humans (Moss et al., 1996). The aim of the present study was to compare the immunogenicity and protective efficacy of two vaccinia viruses, the WR strain and MVA, which express the measles H and F proteins, in infant monkeys in the presence of neutralizing antibody. MV neutralizing antibodies and CTL responses were measured after vaccination in the presence or absence of MV immune globulin (MIG) or natural maternal antibody. Differing levels of protection were observed after a respiratory-mucosal challenge with wild-type MV.

RESULTS

Experimental design and humoral immune responses after vaccination

As shown in Fig. 1, infant monkeys were divided into groups that received 0, 3.5, or 7 ml of MIG 48 h prior to vaccination. At 48 h, the geometric mean neutralizing antibody titer (GMT) in the blood was 160 in Group 4, which received 3.5 ml MIG (range 80–320), and the GMT was 452 in Group 5, which received 7 ml MIG (range 320–2560, Table 1). The GMTs achieved after administration of MIG were similar to the GMT of 226 (range 80–2560) measured in eight monkeys with maternally derived antibody (Table 2). At the same 48 h time point (day of vaccination), sera from six monkeys that received 3.5 or 7 ml MIG were also measured by the conventional plaque reduction neutralization method, and a GMT of 2466 was calculated (range 1393–3734). These titers are in the range for human newborns with maternal antibody (range 32–32,768; Sato et al., 1979).

The infant monkeys were vaccinated by the intradermal (id) route with either WR H&F or WR β gal or by the intranasal (i.n.) and intramuscular (im) routes with MVA H&F or MVA control. Humoral immune responses to vaccination were evaluated by virus neutralization and by radio-immunoprecipitation (RIP) for antibodies to the H and the nucleoprotein (N). Table 1 shows the titers of neutralizing antibody to MV after vaccination with WR β gal or WR H&F in the presence or absence of MIG. Very low levels of MV neutralizing antibody (titers of 10–20) were detected in the two infants of Group 1 vaccinated

FIG. 1. Experimental design: vaccination of infant macaques with vaccinia recombinants expressing MV H and F proteins in the presence or absence of MIG or maternal antibody.
with WR β gal in the absence of MIG. In another two control infants in Group 2, the subcutaneous (sc) inoculation of 7 ml MIG induced titers of 320 and 160 that waned to an undetectable level at 20 weeks, the time of challenge. In contrast, these infant monkeys developed a strong antibody response to vaccinia virus (data not shown). In Group 3 monkeys vaccinated with WR H&F without MIG, MV neutralizing antibody developed after vaccination, and the monkeys had a high titer of antibody at 20 weeks except for Monkey 30310, with a titer of 40. Only two of five monkeys in this group had a rise in antibody titer after the boost at 8 weeks. Antibody to the H protein but not to the N protein, which was absent from the vaccine, was detected by RIP (Fig. 2, Monkey 30989). However, in monkeys vaccinated in the presence of 3.5 or 7 ml MIG, Groups 4 and 5, neutralizing antibody titers waned over the vaccination period even after boosting. This was also demonstrated by RIP (Fig. 2, Monkeys 30991 and 30997). MV neutralizing antibodies were not detected or barely detected in these animals at 20 weeks, and there was no difference in antibody response to vaccination between the monkeys receiving 3.5 or 7 ml MIG. Also, infants that had maternal antibody at the time of vaccination, either low to medium (Group 6) or high titer (Group 7), had waning neutralizing titers in the same ranges as those monkeys that received 3.5 or 7 ml MIG (Table 2).

Immunoprecipitation experiments confirmed the results of the neutralization assays (Fig. 2). In the monkeys vaccinated with WR H&F in the absence of MIG, antibody to H was easily detected by RIP on the day of challenge. In contrast, the amount of H that could be precipitated by

TABLE 1
Neutralizing Antibody Titers to Measles Virus in Monkeys Immunized with Recombinant Vaccinia (WR) in the Presence or Absence of MIG

<table>
<thead>
<tr>
<th>Reciprocal neutralizing antibody titer in immunized monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (WR βgal)</td>
</tr>
<tr>
<td>Group 2 (WR βgal + MIG 7 ml)</td>
</tr>
<tr>
<td>Group 3 (WR H&amp;F + PBS or NMS)</td>
</tr>
<tr>
<td>Group 4 (WR H&amp;F + 3.5 ml MIG)</td>
</tr>
<tr>
<td>Group 5 (WR H&amp;F + 7 ml MIG)</td>
</tr>
<tr>
<td>wks&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIG, PBS, or NMS (normal monkey serum) was inoculated at 48 h prior to immunization at Week 0. Monkeys were boosted at 8 weeks and challenged at Week 20.

<sup>b</sup> Monkey number.

<sup>c</sup> 2 weeks postchallenge.

TABLE 2
Neutralizing Antibody Titers to Measles Virus in Monkeys Immunized with Recombinant Vaccinia (WR) in the Presence of Maternal Antibody

<table>
<thead>
<tr>
<th>Reciprocal neutralizing antibody titer in monkeys immunized with WR H&amp;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 6 (With low/medium titer maternal Ab)</td>
</tr>
<tr>
<td>Group 7 (With high titer maternal Ab)</td>
</tr>
<tr>
<td>wks&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIG or PBS was inoculated at 48 h prior to immunization at Week 0. Monkeys were boosted at 8 weeks and challenged at Week 20.

<sup>b</sup> Monkey number.

<sup>c</sup> 2 weeks postchallenge.
Sera from monkeys that were vaccinated in the presence of maternal antibody or MIG decreased over time and little antibody to H was detectable on the day of challenge. Since N was not present in the vaccine, antibody to N in the postchallenge sera provided an indicator for replication of the challenge virus. In monkeys vaccinated with WR H&F in the absence of MIG or maternal antibody, relatively little N was precipitated by the postchallenge serum sample. Sera from monkeys vaccinated in the presence of MIG or maternal antibody precipitated large amounts of N due to replication of the challenge virus (Fig. 2).

Table 3 shows the titers of neutralizing antibody to MV after vaccination with recombinant MVA in the presence of 0 or 7 ml MIG. In the MVA control monkeys (Groups 8 and 9), antibody titers were similar to those of WR β-gal-vaccinated monkeys. No neutralizing antibody response to MV was induced, a decay of transferred measles antibody was observed, and there was little detectable neutralizing antibody at 20 weeks. In contrast, monkeys vaccinated with MVA H&F in the absence of MIG (Group 10) developed MV-specific antibody after vaccination, and the antibody titers increased after boosting in two of four animals. All animals had a neutralizing antibody titer $\geq 1:80$ at 20 weeks (Table 3), although these titers were lower in general than those of monkeys vaccinated with WR H&F. A decline in neutralizing antibody titers was demonstrated in monkeys that received 7 ml MIG before MVA H&F vaccination in Group 11 with no response to the booster vaccination.

In contrast to MV neutralizing antibody responses, antibody responses to vaccinia virus were detected in all animals that received MIG or PBS, whether by vaccination with WR or MVA (Table 4). Antibody titers to vaccinia virus increased after boosting with MVA H&F, but these titers returned to the levels before boosting, which were similar to those of monkeys vaccinated with WR H&F. No antibody response to MV was detected in Monkey 30330.

![Image](image-url)

**FIG. 2.** Immunoprecipitation of MV H and N proteins by sera from vaccinated monkeys. Six monkeys' sera are shown by vaccine group: Monkey 30998 in Group 1 vaccinated with WR βgal, Monkey 30989/Group 3/WR H&F, Monkeys 30991 and 30997/Group 4/WR H&F + 3.5 ml MIG, Monkey 31000/Group 7, and Monkey 31026/Group 6/WR H&F + maternal antibody. Four lanes are shown for each monkey corresponding to prevaccination (and before administration of MIG), 2 weeks postboost, day of challenge, and 4 weeks postchallenge. The last two lanes at right show precipitation of H and N with monoclonal antibodies specific for each protein. Note that a nonspecific band migrates with the N protein.

| TABLE 3 |
| Neutralizing Antibody Titers to Measles Virus in Monkeys Immunized with Recombinant Vaccinia (MVA) in the Presence or Absence of MIG |

<table>
<thead>
<tr>
<th>Reciprocal neutralizing antibody titers in immunized monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 8 (MVA)</td>
</tr>
<tr>
<td>wks$^a$</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>22$^c$</td>
</tr>
</tbody>
</table>

$^a$ MIG or PBS was inoculated at 48 h prior to immunization at Week 0. Monkeys were boosted at 8 weeks and challenged at Week 20.

$^b$ Monkey number.

$^c$ 2 weeks postchallenge.
at 2 weeks after challenge (Table 1). All other animals developed strong antibody responses to MV after challenge (Tables 1–3).

CTL responses to MV after vaccination in the presence or absence of MIG

Peripheral blood mononuclear cells (PBMC) were collected from all monkeys at Weeks 0, 8, 10, and 20 for the study of MV-specific CTL activity. No CTL response to MV was detected before vaccination, and there were no detectable CTL responses to MV throughout the entire vaccination period in monkeys vaccinated with WR β gal or the MVA control (Groups 1, 2, 8, and 9, Figs. 3 and 4). In Group 3 animals vaccinated with WR H&F without MIG, three of five animals had CTL responses to MV at 8 weeks postvaccination, and five of five had MV-specific

### TABLE 4

<table>
<thead>
<tr>
<th>wks</th>
<th>Group 3 (WR H&amp;F)</th>
<th>Group 5 (WR H&amp;F + 7ml MIG)</th>
<th>Group 10 (MVA H&amp;F)</th>
<th>Group 11 (MVA H&amp;F + 7 ml MIG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30305</td>
<td>30308</td>
<td>30310</td>
<td>30310</td>
</tr>
<tr>
<td>8</td>
<td>30327</td>
<td>30328</td>
<td>30330</td>
<td>30332</td>
</tr>
<tr>
<td>10</td>
<td>30273</td>
<td>30280</td>
<td>30281</td>
<td>30282</td>
</tr>
<tr>
<td>20</td>
<td>30297</td>
<td>30298</td>
<td>30319</td>
<td>30326</td>
</tr>
</tbody>
</table>

*MIG, PBS, or NMS (normal monkey serum) was inoculated at 48 h prior to immunization at Week 0. Monkeys were boosted at 8 weeks and challenged at Week 20.

*Monkey number.

![FIG. 3. MV-specific CTL responses in monkeys immunized with WR vaccinia recombinants. The time point is Week 10, 2 weeks after the second immunization.](image-url)
CTL activity at 2 weeks postboost and at 20 weeks (Fig. 3, Table 5). In the monkeys that received 3.5 ml MIG before vaccination with WR H&F (Group 4), three of five developed CTL activity after vaccination and boosting, and in the monkeys receiving 7 ml MIG before vaccination in Group 5, two of four developed CTL responses to MV (Fig. 3, Table 5). Three of eight monkeys with maternal antibody at the time of vaccination developed CTL responses after vaccination or boosting (Groups 6 and 7, Table 5). Monkeys vaccinated with MVA H&F had results similar to those monkeys vaccinated with WR H&F . If the animals were not given MIG before vaccination (Group 10, Fig. 4), four of four had CTL responses after vaccination and boosting, but only one of four monkeys in Group 11 had detectable CTL activity to MV after receiving MIG (Fig. 4, Table 5).

Clinical signs and viral loads after MV-Davis challenge

All the monkeys were inoculated i.n. with MV-Davis at 20 weeks after the first vaccination. All of the monkeys vaccinated with WR β-gal or the MVA control developed skin rashes between Days 8 and 12 after MV challenge (Groups 1, 2, 8, and 9 and Table 5). However, monkeys that were vaccinated with WR H&F or MVA H&F after receiving 0 or 3.5 ml MIG (Groups 3, 4, and 10) remained healthy and no skin rashes were observed. Two of four monkeys developed skin rashes after MV challenge in Groups 5 and 11, vaccinated with WR H&F or MVA H&F after receiving 7 ml MIG (Table 5). Three of eight monkeys in Groups 6 and 7 that had maternal antibody at the time of vaccination developed skin rashes after MV challenge (Table 5). Skin rashes correlated positively with viral loads in PBMC on Days 7 or 14 after challenge and negatively with CTL activity prior to challenge (see the statistical analysis below).

PBMC on Days 7, 14, and 28 after MV challenge were cocultured with Raji cells to quantitate MV viremia. All four WR β-gal-vaccinated monkeys (with and without MIG, Groups 1 and 2) had a peak of viremia on Day 7, then the viral load declined on Day 14, and MV was not detected on Day 28 (Fig. 5A). This viremia kinetics is similar to that of naive animals inoculated with measles virus (Zhu et al., 1997). No viremia was detected in the five animals of Group 3 vaccinated with WR H&F without MIG (Fig. 5B); in Group 4, one of four monkeys vaccinated with WR H&F with 3.5 ml MIG had no detectable virus in PBMC postchallenge (pc), and the other three infants had low and transient viremia on Day 7 (Fig. 5B). In monkeys vaccinated with WR H&F in the presence of 7 ml MIG (Group 5), there was no detectable viremia pc in Monkey 30330, which had a strong CTL response after vaccination; the other three monkeys had high viral loads which were similar to those of WR β-gal-vaccinated monkeys (Fig. 5B). Thus a dose response to MIG was suggested by the kinetics and peak of viral loads after challenge. The eight monkeys in Groups 6 and 7 with maternal antibody at the time of vaccination had viral loads that were similar to the monkeys vaccinated in the presence of 3.5 or 7 ml MIG; five of eight had high viral loads and only one monkey had no detectable viremia after MV challenge (Table 5).

In the MVA control monkeys of Groups 8 and 9, the kinetics of viremia pc were similar to those of the WR β-gal monkeys (Fig. 6A). Vaccination with MVA H&F, in the absence of MIG (Group 10), resulted in one of four animals with no detectable viremia; the other three monkeys had transient viremia on Day 7 pc, which was 2 logs₁₀ lower than that of the MVA controls (Fig. 6B). One of four monkeys receiving 7 ml MIG before vaccination with MVA H&F (Group 11) had transient viremia on Day 7 pc, and the other three monkeys had high viral loads which were similar to those of the MVA controls (Fig. 6B).

Summary analysis of data (Table 5)

Animals with preexisting antibody at the time of vaccination were significantly less likely to develop a detectable CTL response (P = 0.004) and neutralizing antibody (P = 0.0001) postimmunization, than animals having no preexisting antibody. The postchallenge peak viral load was significantly reduced in animals having no preexisting antibody at the time of vaccination compared to those with low titers (P = 0.0135) or high titers (P = 0.0002) of preexisting antibody. Although there was an obvious overall trend toward increased peak viral load with increasing levels of preexisting antibody at the time of vaccination, the difference in viral load between
A significant inverse relationship was found between the presence of a prechallenge CTL response and postchallenge skin rash, both for all animals, regardless of prechallenge neutralizing antibody status ($P < 0.0001$) as well as for animals without prechallenge neutralizing antibody titers ($P = 0.001$). There were four infant monkeys that had neither neutralizing antibody on the day of challenge nor a detectable MV-specific CTL response prior to challenge that were still protected from high viral loads or skin rash upon pathogenic virus challenge (Monkeys 31024 and 31025 in Group 4, Monkey 31000 in Group 7, and Monkey 30298 in Group 11, Table 5). This suggests that there are other protective immune responses to MV that we did not TABLE 5

Summary of Immune Responses Prior to MV Challenge and Viral Load and Skin Rash after Challenge

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Vaccine ± Ab</th>
<th>Monkey No.</th>
<th>Neutralizing antibody</th>
<th>CTL</th>
<th>MV viral load</th>
<th>Skin rash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WR – βgal + PBS</td>
<td>30988</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+ 7 ml MIG WR H&amp;F</td>
<td>30999</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+ PBS or NMS</td>
<td>31005</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+ 3.5ml MIG</td>
<td>30305</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>+ 7 ml MIG maternal Ab</td>
<td>30306</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+ low/medium-titer maternal Ab</td>
<td>30307</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+ high-titer maternal Ab</td>
<td>30308</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>MVA + PBS</td>
<td>30309</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+ 7 ml MIG MVA H&amp;F</td>
<td>30310</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>+ PBS</td>
<td>30311</td>
<td>–</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>+ 7 ml MIG</td>
<td>30312</td>
<td>+</td>
<td>+</td>
<td>+ + +</td>
<td>+</td>
</tr>
</tbody>
</table>

a Neutralizing antibody titer on the day of challenge: (−) ≤ 20 reciprocal titer, (+) > 20 and ≤ 160, (+++) > 160.

b Measles-specific CTL response detected prior to challenge.

c Viral load after MV challenge: (−) not detected, (+) < 10^3 TCID_{50}/10^6 cells on Day 7 after challenge, (+++) ≤ 10 TCID_{50}/10^6 cells on Day 14 and (++++) > 10^3 TCID_{50}/10^6 cells on Day 7 and > 10 TCID_{50}/10^6 cells on Day 14.

groups with low and high titers of preexisting antibody was not significant at the alpha = 0.05 level ($P = 0.0942$). A significant inverse relationship was found between the presence of a prechallenge CTL response and postchallenge skin rash, both for all animals, regardless of prechallenge neutralizing antibody status ($P = 0.0001$) as well as for animals without prechallenge neutralizing antibody titers ($P = 0.001$).
measure and that they are operative in the presence of maternal antibody. These immune responses could include antibody dependent cellular cytotoxicity, delayed type hypersensitivity, or antiviral CTL or neutralizing antibody below the level of detection.

**DISCUSSION**

The major observations obtained from this study are (1) that both WR and MVA vaccinia vectors are safe in newborn rhesus monkeys and that they can immunize newborns to recombinant viral proteins with the induction of both neutralizing antibody and CTL responses, (2) that either maternal or passively transferred MV neutralizing antibody effectively blocks the humoral response to H and F expressed by vaccinia virus in a prime-boost regimen, (3) that the frequency of CTL responses to MV in infants is reduced by preexisting measles antibody, and (4) that some level of protective immunity, probably a cell-mediated response, prevents the measles skin rash despite the inhibitory effect of antibody at the time of immunization. Direct comparisons of the WR and MVA strains of vaccinia virus as vaccine vectors revealed similar levels of humoral and cellular immunity in mice (Ramirez et al., 2000; Sutter et al., 1994). However, in this study, peak levels of MV neutralizing antibody were lower in infant monkeys vaccinated with the MVA vector, and MVA was unable to prevent viremia after challenge. It should be noted that the WR strain of vaccinia is moderately pathogenic in rhesus macaques and that the MVA strain cannot replicate fully in mammalian cells. In contrast to the results reported herein, in another recent study, macaques vaccinated with the same recombinant measles MVA in the presence of MV neutralizing antibody did respond to a booster vaccination at 1 month, and these animals were protected from systemic infection 1 year after vaccination (Stittelaar et al., 2000). However, that study modeled the vaccination of 6- to 9-month-old infants with lower levels of maternal antibody than newborns (Sato et al., 1979) and with a more mature immune system.

In infant monkeys with preexisting antibody, it is striking that the neutralizing antibody response was inhibited after the booster immunization at 8 weeks when MIG or maternal antibody had declined to low levels. In the

![FIG. 5. MV viral loads after challenge in monkeys immunized with WR vaccinia recombinants. In the top graph, the TCID₅₀ end-point titers of MV are shown for monkeys that were immunized with control WR βgal in the absence (closed symbols, Group 1) or presence of MIG (open symbols, Group 2). In the bottom graph, viral titers are shown for monkeys that were immunized with WR H&F in the presence of 7 ml MIG (open symbols, 4 monkeys of Group 5) or 3.5 ml MIG (closed symbols, 5 monkeys of Group 4). The five monkeys of Group 3 that did not receive MIG had no detectable viremia and their data are not shown. The symbol legends indicate individual monkeys in each group.](image1)

![FIG. 6. MV viral loads after challenge in monkeys immunized with MVA vaccinia recombinants. In the top graph, the TCID₅₀ end-point titers of MV are shown for the monkeys that were immunized with the control MVA in the absence (closed symbols, Group 8) or the presence of MIG (open symbols, Group 9). In the bottom graph, viral titers are shown for monkeys that were immunized with MVA H&F in the absence (closed symbols, Group 10) or the presence of MIG (open symbols, Group 11). The symbol legends indicate individual monkeys in each group.](image2)
absence of MIG, a boosted neutralizing antibody response was observed in some infants. Two of five infants immunized with WR H&F (Table 1, Group 3) and two of four infants immunized with MVA H&F (Table 3, Group 10) had fourfold rises in antibody titer after the boost at 8 weeks. Other investigations have shown that systemic immunity to vaccinia virus inhibits the response to a novel recombinant viral protein delivered with a secondary vaccinia exposure (Belyakov et al., 1999; Cooney et al., 1991; Rooney et al., 1988). In these reports, vaccinia recombinants were neutralized immediately after inoculation, and the effectiveness of the vaccine was reduced, but this rapid neutralization could be overcome by inoculating the virus by a mucosal route (Belyakov et al., 1999). Preexisting vaccinia immunity inhibited both humoral and cellular responses to recombinant antigens (Belyakov et al., 1999). In the present study, antibody responses to the vaccinia vector were not boosted at 8 weeks in infants immunized with WR (Groups 3 and 5, Table 4), but there was a large booster response to the MVA vector (Groups 10 and 11, Table 4). This difference may be due to the abortive infection by MVA or to the fact that MVA-immunized infants were inoculated by both a parenteral and a mucosal route.

Maternally acquired antibodies are important in the protection of infants from disease in the first few months of life, but the presence of maternal antibodies at the time of vaccination interferes with responses to several infant vaccines, such as for measles, parainfluenza, and respiratory syncytial viruses (Durbin et al., 1999; Halsey et al., 1985; Siegrist et al., 1988b). We hypothesized that the chimeric expression of measles H and F in a vaccinia vector could overcome the inhibition by maternal antibody, which we proposed was due to viral neutralization. Indeed, the pool of MIG that we used did not neutralize the WR H&F vector in a vaccinia neutralization assay (data not shown), and it is unlikely that MV H and F glycoproteins are expressed on the surface of this recombinant vaccinia virion. Contrary to our hypothesis, preexisting MV antibody reduced the humoral and cellular immune responses to measles H and F antigens expressed by vaccinia virus in vivo. Several similar observations from mice, monkeys, and humans have been reported for MV (Halsey et al., 1985; Kumar et al., 1998; Siegrist et al., 1998a; van Binnendijk et al., 1997) and other paramyxoviruses (Durbin et al., 1999; Murphy et al., 1988). This blockade, more effective against the priming of B cells than of T cells, may be due to immune complex formation at the site of vaccinia replication and the subsequent degradation of antibody-complexed H and F proteins by tissue macrophages. Alternatively, MV antibody might downregulate the expression of the H and F at the cell surface (Fujinami and Oldstone, 1979).

The MIG pool that was used in the present experiment has serum proteins other than immunoglobulins that may have influenced the outcome of vaccination. And the immunoglobulin components of MIG and maternally acquired antibody are not the same; IgG 1 and IgG 3 have high-affinity receptors in the placenta, and they are preferentially transferred to the fetus (McNabb et al., 1976). Eight infant monkeys with low- to high-titer maternal antibodies (average neutralizing antibody titer of 160) were vaccinated with WR H&F, and no detectable MV-specific humoral responses were induced. CTL responses were detected in three of these eight monkeys, similar to the number of monkeys vaccinated with WR H&F after receiving 3.5 or 7 ml MIG. Thus, at the levels of immune response, viremia after pathogenic virus challenge and clinical disease, MIG closely mimicked natural maternal antibody. Further, there was no inhibitory effect on immune responses to vaccination by a pool of normal monkey sera that was measles seronegative (2 infant monkeys in Group 3, Table 1).

In these experiments, infant monkeys (1–2 weeks old) were immunocompetent: they developed both humoral and cellular immunity after vaccination, but we do not have data from juvenile or adult monkeys to compare immune responses quantitatively. Recently, Gans et al. reported that 6-month-old human infants have a deficiency in the humoral immune response but not in a cellular immune response to measles vaccination by comparison with 9- or 12-month-old infants (Gans et al., 1998). Similar observations were made for respiratory syncytial virus infection of infants (Murphy et al., 1986). But immaturity of the infant immune system, particularly at the humoral level, would not preclude successful immunization of newborns against viral pathogens like MV. In this study, the inhibition of humoral responses by preexisting antibody did not preclude protection from the measles skin rash.

Although neutralizing antibody is a reliable sign of vaccine-induced immunity, cellular immunity is also essential to recovery from measles (Jaye et al., 1998a). Children with congenital agammaglobulinemia that contract measles develop protective immunity without detectable antibody after infection (Good and Zak, 1956). Recently, it was shown that vaccinated children with no detectable antibody response are protected from measles and it was suggested that these children may have cellular immunity that provides the protection (Carson et al., 1995; Samb et al., 1995). Although antibody responses to H and F were undetectable in the vaccinated monkeys receiving MIG, by either neutralization assay or by RIP, some of these monkeys developed CTL responses to MV. There are several similar observations both in monkeys and in mice (Siegrist et al., 1998b; van Binnendijk et al., 1997). In these reports, antibody responses were inhibited, but cellular immunity was not only partially inhibited. And those monkeys that developed cellular immunity without humoral immunity after vaccination had very low cell-associated viremia after challenge with wild-type virus (van Binnendijk et al.,
1994). As recently proposed (Seiler et al., 1998), the present results demonstrate that even though infant vaccines given in the presence of maternal antibody may not be able to induce a detectable antibody response or to prevent viral replication, they may stimulate a cellular response capable of clearing infection and preventing severe disease.

**MATERIALS AND METHODS**

Construction of WR and MVA recombinant vaccinia viruses expressing MV H and F proteins

A recombinant WR strain of vaccinia virus expressing both the H and F proteins of the Edmonston strain of MV (WR H&F) was constructed as previously described (Tamin et al., 1994). In this recombinant virus, H was inserted into the TK gene under the control of the P7.5 promoter and F was inserted into the vaccinia HA gene and expressed using the M1 promoter. Construction of MVA H&F, a double-recombinant expressing the H and F of the Edmonston strain of MV, was described elsewhere (Stittelaar et al., 2000). Briefly, the H and F genes were placed under control of the modified H5 and P7.5 promoters, respectively, and inserted into deletions II and III of the MVA genome, respectively. Both MVA and MVA H&F were propagated in chicken embryo fibroblasts as described (Sutter et al., 1994).

Passive transfer of MIG prior to vaccination

MIG is a pool of heated-inactivated sera from monkeys that contracted measles during an outbreak amongst juvenile monkeys at the California Regional Primate Research Center in 1995. The reciprocal neutralizing antibody titer of MIG is 5120 by 50% end-point titration. MIG (3.5 or 7 ml) was administered to infant rhesus monkeys by SC inoculation 48 h prior to vaccination. Control infant monkeys were inoculated sc with either 7 ml of phosphate-buffered saline (PBS) or pooled, heat-inactivated normal monkey sera (NMS) that was negative for measles antibody by IgG ELISA (Zhu et al., 1997).

Vaccination with WR and MVA recombinant viruses

Colonby-bred male and female newborn rhesus macaques (Macaca mulatta), seronegative for simian type D retroviruses, simian T-cell lymphotropic virus, and simian immunodeficiency virus, were housed in accordance with the American Association for Accreditation of Laboratory Animal Care. This macaque colony is routinely vaccinated against measles (Attenuavax, Merck and Co., Inc., Westpoint, PA), and ~25% of newborn monkeys have maternal measles-neutralizing antibody. The investigators adhered to the guidelines of the Committee on Care and Use of Laboratory Animals, National Resources Council. Infant monkeys (1–2 weeks old, body weight ~0.5 kg), after assaying for measles-neutralizing antibody, were vaccinated with either recombinant WR or MVA (Fig. 1). In the WR groups, all animals were vaccinated id with 1 \times 10^6 plaque-forming units (pfu), an optimal route and dose for this virus in infant monkeys (M. McChesney, unpublished data). As shown in Fig. 1, four control monkeys were vaccinated with WR expressing beta galactosidase (WR-β gal) in the presence of 7 ml MIG (n = 2) or PBS (n = 2). Fourteen infants were vaccinated with WR H&F in the presence of PBS (n = 3) or NMS (n = 2). 3.5 ml (n = 5) or 7 ml MIG (n = 4). Another eight infant monkeys that had maternal antibody at a low to moderate neutralizing titer (80–160, n = 5) or a high titer (320–2560, n = 3) were vaccinated with WR H&F. In the MVA groups, all monkeys were vaccinated with 1 \times 10^6 pfu by both the im and i.n. routes. Four control monkeys were vaccinated with parental MVA in the presence of 7 ml MIG (n = 2) or PBS (n = 2). Eight infants were vaccinated with MVA H&F in the presence of 7 ml PBS (n = 4) or 7 ml MIG (n = 4, Fig. 1). All of the animals were boosted with the same recombinant viruses and doses at 8 weeks after the first vaccination, but MIG was only administered before the first vaccination.

Measurements of MV neutralizing antibodies and antibodies to H and N proteins by radio-immunoprecipitation (RIP)

MV neutralizing antibody was measured by viral antigen reduction in Vero cells in 96-well plates as described previously (Zhu et al., 1997). Neutralizing antibody titers were calculated as the highest dilution showing 50% reduction in optical density of six control wells that contained virus without serum. Selected sera were also tested for viral neutralization by the plaque reduction method (Albrecht et al., 1981). Antibodies to measles H and N proteins were detected by RIP as previously described (Tamin et al., 1994), with a single exception. The infected-cell pellet was resuspended in RIP buffer (1% sodium deoxycholate, 1% Triton X-100, 0.2% SDS, 150 mM NaCl, and 50 mM Tris–HCl at pH 7.4).

Measurement of MV-specific CTL

PBMC were isolated from heparinized blood and cryopreserved in liquid nitrogen until use. To measure measles CTL responses, PBMC were re-stimulated in vitro with MV antigen, followed by a 4-h chromium release assay as previously described (Zhu et al., 1997). The lysis of mock-infected and measles virus-infected autologous B cell lines was measured and the percent specific lysis was calculated as: 100 \times [{\text{experimental release} - \text{spontaneous release}}]/{\text{maximal release} - \text{spontaneous release}}]. Specific lysis was considered positive if the lysis of infected targets was greater than twofold above the lysis of mock-infected targets and if it was ≥10%.
Measurement of antibodies to vaccinia virus by ELISA

Vaccinia virus antibodies were measured in plasma samples from immunized animals as previously described (Hirsch et al., 1996) with the following modifications. PBS containing 5% milk and 0.5% Tween 20 was used for blocking. The substrate, BM Blue POD (Boehringer–Mannheim) was used according to the manufacturer’s instructions.

Challenge with pathogenic MV: clinical signs and MV viral load

At 12 weeks after boosting, the monkeys were challenged by i.n. inoculation of 10⁶ tissues culture infectious doses 50% (TCID₅₀) of the Davis 87 isolate of measles virus grown in rhesus PBMC (McChesney et al., 1997). The animals were monitored daily for anorexia, depression, coughing, dysentery, and skin rash. They were bled on Days 7, 14, and 28 pc. Quantitation of viral load in PBMC by end-point dilution coculture with Raji cells was performed as previously described (Zhu et al., 1997). The cultures were monitored for cytopathic effect and scored by an indirect immunofluorescence assay using the KK2-mAb to measles N protein (Bellini et al., 1986). The viral titer was expressed as the TCID₅₀ per 10⁶ cells using the method of Reed and Muench (1938).

Statistical analysis

For purposes of analysis, CTL response, antibody status, and skin rash were considered as dichotomous variables. Fisher’s exact probability test was used to analyze the relationship between antibody status at the time of vaccination and postimmunization CTL and neutralizing antibody responses and the relationship between prechallenge CTL response, with or without neutralizing antibody, on postchallenge skin-rash development. Differences in postchallenge peak virus load among animals with no, low, or high preexisting antibody (natural maternal or MIG) at the time of vaccination were analyzed using the Kruskal-Wallis one-way ANOVA by Ranks. Post hoc comparisons were performed using the Wilcoxin-Mann-Whitney test.

ACKNOWLEDGMENTS

We thank Linda Hirst and the nursery staff, Irene Williams for performing the measles plaque reduction neutralization assay, and Yves Riviere for critical discussion. This publication was supported by NIH Grant RR-00169 and by Cooperative Agreement U50/CCU913348 from the Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the CDC.

REFERENCES


