MV nine months later. No evidence was found in these experiments of histopathological features consistent with ''atypical measles'' (63,67). Future studies of interest for these vaccines in nonhuman primates include immunogenicity in the presence of maternal antibodies and analysis of immunosuppression.

Biodistribution, Integration, and Toxicology Studies

Studies assessing the biodistribution of the DNA vaccines were undertaken to detect any evidence of DNA integration (55). pMSIN-H and pMSINH-FdU were administered ID to New Zealand white rabbits at their intended clinical dosage levels via the Biojector 2000 needle-free injection system and biodistribution was monitored during a 60-day period. A single dose of 1.76 mg of pMSIN-H or 1.84 mg of pMSINH-FdU had no effect on mortality, clinical and cageside observations, body weights, body weight changes, and food consumption. The only vaccine-related effects observed were minimal transient erythema, edema, and inflammation confined to the injection site. The plasmids persisted at the injection site skin and subcutis, injection site muscle, and (to a much lesser degree) in the popliteal lymph nodes that drain the injection sites for the duration of the study (55). Integration studies showed no evidence of plasmid integration into the rabbit host genome.

To assess potential toxicological effects, New Zealand white rabbits were primed ID with pMSIN-H (1.76 mg), pMSINH-FdU (1.84 mg), or phosphate buffered saline (PBS) (control article), on study days 1, 29, and 57 (55). Some animals received a subcutaneous (SC) injection (boost) of 0.5 ml of PBS or $\sim 10^3$ tissue culture 50% infective dose ($TCID_{50}$) of the EZ measles vaccine. Subgroups were euthanized at different time points prior to and after the boost, and no effects were found on mortality, clinical observations, cageside observations, body weights, body weight changes, food consumption, clinical pathology, organ weights, or organ weight ratios. Increased frequency, score, and recovery time of dermal Draize observations at the pMSIN-H, and pMSINH-FdU injection sites were observed, which correlated with injection site gross findings (red discoloration on study day 60 only) and histopathological findings of inflammation that recovered with time (55). Both Sindbis-based vaccine plasmids were immunogenic in rabbits; as observed in other species, pMSIN-H elicited higher PRN titers.

Phase I Clinical Studies

The extensive preclinical data demonstrating the safety, immunogenicity, and efficacy of the Sindbis replicon measles vaccines led to filing of a new investigational drug application (IND) to support the performance of a phase I clinical trial. On the basis of the superior immunogenicity and efficacy of pMSIN-H in the extensive preclinical animal model experiments, it was the favored DNA vaccine candidate to move forward in clinical trials. Nevertheless, on the assumption that humans might respond differently, we elected also to study pMSINH-FdU vaccine, in addition to pMSIN-H, at least in phase I.

We undertook conduct of the phase I trial of the DNA vaccines in healthy adults of ages 18 to 45 years living in the United States who participated in a randomized, double-blind, placebo-controlled, dose-escalating, outpatient study to assess three dosage levels of approximately 200, 400, and 800 μg of each vaccine in a stepwise fashion. At each dosage level, 20 subjects were allocated to one of four groups to receive two doses of vaccine and one dose of placebo on days 0, 28, and 56, as follows: (*i*) pMSIN-H, pMSIN-H, placebo ($n = 5$); (*ii*) placebo, pMSIN-H, pMSIN-H ($n = 5$); (*iii*) pMSINH-FdU, pMSINH-FdU, placebo ($n = 5$); or (*iv*) placebo, pMSINH-FdU, pMSINH-FdU($n = 5$). The vaccines were administered ID using Biojector 2000 (Kotloff and Levine, personal communication). The purpose of this study was to generate preliminary safety data prior to considering the evaluation of the safety and immunogenicity of this regimen in developing countries, where the ultimate target population resides. Since routine infant immunization in sub-Saharan Africa involves contacts at 6, 10, and 14 weeks of age, the ultimate goal is to administer one of these DNA vaccines at 6 and 10 weeks of age as the priming immunogen, followed by a dose of currently licensed attenuated measles vaccine as the boosting immunogen at 14 weeks of age. This strategy, if successful, would allow an infant to be immunized before the window of vulnerability opens at $\sim$16 weeks of age.

ALTERNATIVE MEASLES VACCINE CANDIDATES

A handful of DNA vectors have been proposed as potential measles vaccine candidates and shown to prime immune responses in juvenile (63,68,69) and very young infant macaques (41,42) and to confer varying degrees of protection against wild-type MV challenge. Immune responses, however, were quite variable. When administered to infant macaques, they either failed to develop neutralizing antibodies (42) or generated poor responses (70). Different approaches have been described to improve the immunogenicity of these vaccines including the use of codon-optimized genes and adjuvants such as vaxfectin (70) and plasmid-encoded cytokines (42). Other genetic vaccine candidates explored include recombinant viral vectors such as alphavirus replicon particles (71), parainfluenza (72), and vaccinia virus expressing MV antigens (69,73,74).

Subunit and epitope-based vaccines have been described (75,76), but they elicit limited and short-term immunity. A proteosome-MV H and F vaccine administered to juvenile rhesus macaques alone in three consecutive immunizations or as a boost following priming with Sindbis virus measles DNA vaccines conferred full protection against MV challenge (54). Despite their initial promise in preclinical studies, none of these approaches has yet been investigated in humans.

CONCLUSION

Despite the overall progress achieved with mass immunization campaigns, in several countries in sub-Saharan Africa (e.g., Niger, Chad), measles mortality in young children remains a serious health problem (12).

A Sindbis replicon measles DNA vaccine encoding measles H was shown to be highly immunogenic and to induce protective immunity in nonhuman primates. Among alternative vaccine strategies for young infants, this is the most advanced, having been tested in a phase I study that is nearing completion. The ultimate goal is to administer such a new measles vaccine candidate at 6 and 10 weeks of age as the priming immunogen, followed by the currently licensed attenuated measles vaccine as the boosting immunogen at 14 weeks of age. This strategy, if successful, would provide a means to protect infants during the critical window of vulnerability.