Response to Immunization with a Monovalent Measles Vaccine in Pigtailed Macaques

B.J. Weigler*, DVM, MPH, PhD, DACLAM, DACVP; L. Kuller, BS; M.B. Agy, PhD; and C.E. Hotchkiss, DVM, PhD, DACLAM
Washington National Primate Research Center
University of Washington, Seattle, WA 98195-7303
*Corresponding Author: bweigler@wanprc.org

ABSTRACT

We studied the safety and immunogenicity of a live, attenuated, monovalent, Edmonston-Zagreb strain measles vaccine (“M-vac”, Serum Institute of India) that is commonly used overseas for possible use in protecting pigtailed macaques (Macaca nemestrina) against a measles outbreak. The vaccine meets WHO requirements and contains ≥1000 CCID₃₀ virus particles/dose. Anecdotal reports from other centers using M-vac under a CDC special import license indicated that it was safe and immunogenic in cynomolgus and rhesus macaques. Cost considerations and absence of other viruses in the formulation made this an attractive candidate for evaluation. We explored if binding (MFI) and serum neutralizing antibodies (SNAB) formed at levels considered protective against infection and to assure safety in this species. The study involved two phases; a 2-week follow-up acute safety study (Phase I) and a longer-term follow-up study (Phase II) where safety and immunogenicity were monitored over 7-months post-immunization. In Phase I, five M. nemestrina (age 3 – 20 years; 2 F, 3 M) were used to provide baseline and post-immunization antibody titers, CBCs with lymphocyte subset analysis, clinical chemistry panels, gross necropsy, and histopathology of the immunization site. Each animal was scored daily based on eight body-system clinical parameters. In Phase II, 36 M. nemestrina (age 1 – 12 years; 28 F, 8 M) were immunized then monitored for antibody responses two and seven months later; clinical scores, CBCs, and clinical chemistry panels were also obtained. Collected serum samples were tested against two measles antigen formats, MRC-5 human diploid cells used to propagate the vaccine, and negative and positive controls. The SNAB assays were done in Vero cells with a molecular clone of attenuated Moraten strain expressing green fluorescent protein using Reed-Muench methods to calculate reciprocal endpoints. Although binding (MFI) Ab responses across assay formats were robust in most animals (86%) within weeks of immunization, neutralizing antibodies were virtually absent in all but two animals (kappa = 0.2). No adverse reactions occurred. These findings provide an excellent example of unanticipated differences that may occur in features of biological response between macaque species, thus emphasizing the importance of validating the predefined outcomes for each primate model selected.

General Background on Measles in NHPs

Measles (rubella virus) outbreaks in nonhuman primates (NHPs) are a direct result of exposure to infected humans or to other NHPs that have been infected by humans. Infection has been documented in Asian Old World monkeys used most frequently in biomedical research, including rhesus, cynomolgus, pigtailed, and Japanese macaques, and has also been reported in various African Old World and New World monkey species. More recently, large epizootics of measles and related morbillivirus infections have occurred in native macaque populations within Asia. Exported animals exposed in their country of origin may pose a serious threat to U.S. colonies. Macaques infected with measles may show nasal discharge, fever, anorexia, facial edema, conjunctivitis, cutaneous rash, respiratory distress, enteritis, and sometimes death. Measles epizootics in research facilities are rare events but can be devastating nonetheless, greatly impacting resources and their ability to meet demand. Measles has an estimated basic reproduction number (R₀) of 12 to 40, meaning that 1 case introduced into a susceptible ( naïve) population will produce, on average, that range of secondary cases. Presently, the only effective methods for primary prevention are prior immunization and strict control over contact sources.

Why This Vaccine?

• Concerns existed regarding cost & availability of an efficacious vaccine.
• NIH National Primate Center’s Breeding Colony Management Consortium organized a multi-site evaluation of “M-vac” monovalent measles vaccine.
• M-Vac is produced by the Serum Institute of India (SII), India’s No. 1 Biotech Company and the World’s 5th largest vaccine manufacturer.
• The SII is one of the largest suppliers of vaccines to UNICEF & PAHO and caters to over 140 countries.
• M-vac is the Edmonston-Zagreb strain, live, attenuated after 22 passages on human diploid cells (MRC-5, human lung fibroblasts).
• Each lyophilized dose (0.5 ml) contains ≥1000 CCID₃₀ live virus particles.
• Manufacturer indicates a single dose will produce lifelong protection.
• Vaccine meets WHO Technical Report Series 840 requirements (1994)
• Distribution of imported vaccine was through CDC to one of the NPRCs.
• Route of administration is subcutaneous - we used the interscapular area.
• Studies involving pigtailed macaques only occurred at the WaNPRC.

Design and Analysis

Phase I Substudy

<table>
<thead>
<tr>
<th>Phase I Substudy</th>
<th>Purpose</th>
<th>Methods</th>
<th>Outcome</th>
<th>Scores</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I Substudy</td>
<td>To test for adverse events from use of M-vac in pigtailed macaques.</td>
<td>Five available M. nemestrina (age 3 – 20 years; 2 F, 3 M) pre-scheduled for WaNPRC’s Tissue Distribution Program enabling necropsy procedures.</td>
<td>Baseline and post-immunization (day 14) antibody titers, CBCs, clinical chemistries, and histopathology of the subcutis immunization sites.</td>
<td>Daily by cage-side experiments based on 8 scored body-systems parameters.</td>
<td>Binding Ab (purified virus vs recombinant (rec-) nucleocapsid vs MRC-5).</td>
</tr>
</tbody>
</table>

Phase II Substudy

<table>
<thead>
<tr>
<th>Phase II Substudy</th>
<th>Purpose</th>
<th>Methods</th>
<th>Outcome</th>
<th>Scores</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II Substudy</td>
<td>Pending no adverse reactions from Phase I, to immunize and monitor M. nemestrina for at least 6 months to evaluate their clinical responses and characterize immunological responses to the M-vaccine.</td>
<td>36 research colony M. nemestrina (age 1 to 12 years; 28, 8 M)</td>
<td>Baseline and post-immunization (2 month &amp; 7 month) antibody titers, CBCs, and clinical chemistries.</td>
<td>Two weeks of daily post-vaccination cage-side exams, as above.</td>
<td>Binding antibody (purified measles Ag, rec-nucleocapsid, MRC-5) &amp; SNAB.</td>
</tr>
</tbody>
</table>

Main Results

Phase I

• All eight body-systems health scores were normal throughout.
• No remarkable findings in CBC or clinical chemistry panels.
• No gross external or internal abnormalities; skin/dermis/subcutis essentially normal.
• Two (of 5) seroconverted in binding Ab assay (highest with use of rec-nucleocapsid).
• One animal had unexplained pre-existing Ab to MRC-5 vaccine host cells.
• Based upon the above, a decision was made to proceed with Phase II.

Phase II

• All eight body-systems health scores were normal throughout.
• No remarkable findings in CBC or clinical chemistry panels.
• Seven (of 36) were unavailable for follow-up at 7 months post-vaccination.
• One (imported) M. nemestrina was seropositive to measles in all assays at all time points, probably indicating natural exposure; no history of prior vaccination.
• 30 seroconverted to binding Ab by 2 months, but it waned in 45% by the 7th month.
• Only two developed SNAB to measles; one of those waned by the 7th month.

References


Acknowledgments: We thank John Yee and Ann Rosehill of the California National Primate Research Center-Davis, CA for performing binding and neutralizing antibody assays. This work was approved by the Institutional Animal Care and Use Committee at the University of Washington. Funding was provided by NIH: 1R01-OD01945.