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Parvovirus B19 VLP Vaccine Manufacturing

Meridian Developed Production Process for Technology Licensed from NHLBI

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A clinical-grade recombinant virus-like particle (VLP) vaccine has been developed to prevent human parvovirus B19 (HPVB19) infection, which can have severe and potentially fatal complications in susceptible populations. The vaccine consists of two viral proteins (VP1 and VP2) in separate baculovirus vectors that are co-infected at the correct multiplicity of infections (MOIs) into *Spodoptera frugiperda* (Sf9) cells and that, upon expression, self-assemble into immunogenic virus-like particles.

HPVB19 replicates principally in human erythrocyte precursors resulting in suppression of erythropoesis and anemia (*Figure 1*). The incubation period is 4 to 20 days with viremia at 6 to 8 days. The healthy host is asymptomatic during viremia with a characteristic rash appearing at approximately 16 days. The virus is shed from the nasopharynx and is spread by respiratory droplets.

In the healthy host, HPVB19 causes fifth disease (erythema infectiosum) and an arthralgia syndrome in adults. In individuals with underlying hemolysis, infections result in transient aplastic crisis (TAC), a temporary cessation of red blood cell production with severe and occasionally fatal anemia. Immunosuppressed and immune-compromised individuals may experience persistent and severe anemia and pure red cell aplasia. Infection during the first and second trimesters of pregnancy can result in hydrops fetalis and fetal loss (miscarriage).

HPVB19 infection has been reported worldwide and occurs year round, although infections in temperate climates are more common in late winter and spring. Current treatment modalities include red blood cell transfusions for patients with TAC, transfusions and immune globulin administration for infected immunocompromised patients, and intrauterine exchange transfusions for hydrops fetalis.

Immunization is a promising strategy to prevent serious parvovirus infection in high-risk groups, as well as in the general population. Studies in the hematology branch of the National Heart, Lung, and Blood Institute (NHLBI) have resulted in the production of recombinant parvovirus capsids, which lack infectious DNA, but retain the immunogenicity of native virions. These empty viral capsids have been utilized in human volunteer studies to elicit neutralizing anti-

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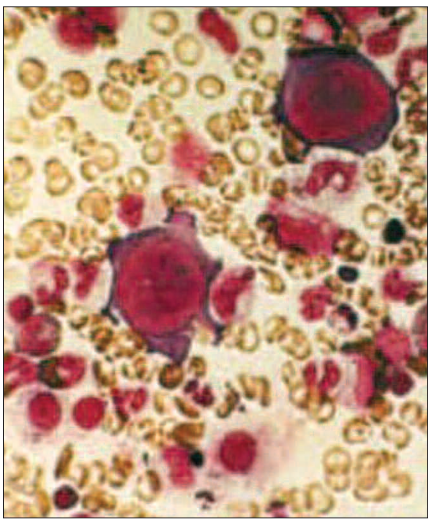

Figure 1. This bone marrow aspirate from an infected patient lacks mature erythroid precursors and has giant pronormoblasts that result from the cytopathic effect of the virus.

Photo courtesy of Dr. O. Caul

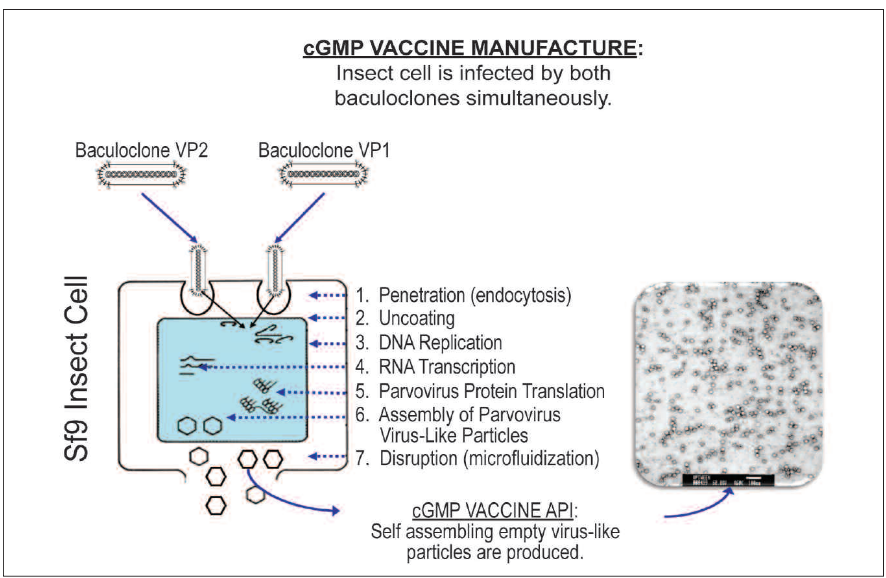

Figure 2. VLP production: Two baculoclones possessing DNA encoding for the VP1 or VP2 capsid proteins are coinfected into Sf9 cells (left). Baculovirus penetration, uncoating, and protein transcription/translation occur subsequently, then expressed proteins assemble into VLPs. On the right is a transmission electron micrograph of empty VLPs.

body responses.

HPVB19 has a small linear, single-stranded DNA genome, which encodes two capsid proteins and a single non-structural protein (NS-1). The major capsid protein, VP2, constitutes about 95% of the capsid structure, the minor capsid protein, VP1, is identical except for an additional 226 amino acids at the amino terminus. Investigators at the NHLBI further showed that neutralizing linear epitopes cluster in the VP1-unique and VP1-VP2 junction regions.

Technology to express VP1 and VP2, derived from a copy of the parvovirus B-19 genome (strain Au) obtained from a child with sickle cell disease and TAC, was developed at the NHLBI. Individual viral proteins were expressed in a baculovirus system and, when recombinant vectors are cotransfected into insect cells, VP1 and VP2 spontaneously assemble into empty parvovirus VLP's.

Production Process

In a CRADA between NHLBI and **MedImmune**, a manufacturing process was developed for the VLP vaccine and multiple animal species were tested. Initial studies showed that enrichment of VLPs by manipulating multiplicity of infection (MOI) for the baculoclones could produce capsids containing increased VP1 (20 to 40%), and that such preparations were effective in promoting neutralizing antibody response.

In the first Phase I clinical study, VLPs formulated with aluminum hydroxide elicited low neutralizing antibody titers. A second Phase I study, conducted using VLPs formulated with the **Novartis** squalene-based MF59c.1 adjuvant yielded excellent neutralizing antibody responses in humans. However, MedImmune ceased development of the vaccine and returned the vaccine rights to the NIH.

Meridian Life Science (www.meridianlifescience.com) was then contracted to manufacture the vaccine under cGMP to support further development by NHLBI. Meridian subsequently licensed the vaccine technology, developed a more robust manufacturing process, and successfully manufactured Phase I materials.

Vaccine Manufacturing

Manufacturing of the HPVB19 VLP vaccine using a two baculovirus system poses some challenges with respect to maintaining the ratios of the two viral proteins and in downstream purification. The process, however, has been sufficiently robust to allow the manufacture of three clinical lots by two different manufacturers, all of which were used in humans. After transfer of the process from the original manufacturer, initial pilot production studies were conducted to demonstrate reproducibility of the process and comparability of the resulting VLPs.

The process was then scaled to a 20 L Wave culture. The Sf9 working cell bank was expanded through a series of shake flask cultures and transferred to a 50 liter WaveBag® (**GE Healthcare**) at 1.5 x 10^6 cells/mL and allowed to expand until 20 liters of culture at a cell density of 1.5 to 2 x 10^6 were obtained. The culture was co-infected with bacVP1 and bacVP2 at an MOI of 1 and 0.5 respectively (*Figure 2*).

The bioreactor was incubated at 26° to 28°C for four days and harvested when the cell viability dropped below 50%. The temperature, pH and rocking speed, and angle were monitored. Cell density and viability were monitored daily. The culture was harvested by centrifugation at 800 x g for 30 minutes after which the fluid was decanted and the cell paste stored at -60° to -80°C.

VLPs were purified by resuspending cell paste in a tris buffer with 1.6 µM leupeptin and microfluidized to prepare a cell lysate. Protocols transferred from the previous industry partner utilized fil-

tration to remove insoluble particles with a single DEAE anion exchange chromatography step.

This filtration step was changed to improve manufacturability and product yields. Lysate was diluted with 20 mM Tris-HCL to reduce salt concentration and subjected to fluidized bed ion-exchange chromatography using Streamline DEAE media (GE Healthcare) in lieu of filtration. Eluate containing VLP's was diluted with 20 mM Tris-HCl to reduce salt concentration and subjected to Fractogel DEAE ion-exchange column chromatography.

After washing with Tris-HCl buffer, the product was eluted with a phosphate buffer and the pH was adjusted for final polishing using Fractogel TMAE ion-exchange chromatography. The vaccine product was eluted and formulated with sucrose and Tween excipients. The final concentrated bulk product was filtered through a 0.22 µ filter.

Dilution of the vaccine to target concentrations of either 10 µg/mL or 100 µg/mL resulted in loss of the drug due to protein adherence to the vial. Formulation studies utilizing **Schott** Plus-1 vials combined with sucrose and Tween 80 minimized loss. Formulation and purification studies required a sensitive and specific method to track the vaccine.

A sandwich ELISA utilizing both polyclonal and monoclonal antibodies specific for HPVB19 was developed that demonstrated a sensitivity of ≤50 ng/mL and variability of under 20% within the range of quantitation (100 ng/mL to 750 ng/mL).

Conclusions

Multiple groups are at risk of potentially life-threatening complications due to human parvovirus B19 infection. Based on the infectious nature of HPVB19 infection in children and the severe potential consequence of transplacental fetal infection, a two-pronged vaccination strategy to manage HPVB19 infections in these populations is recommended.

First, incorporation of a HPVB19 vaccine into the early childhood vaccination schedule could reduce the overall incidence of this virus in the population. Second, a safe vaccine that can elicit protective neutralizing antibodies could be provided to women of child-bearing age as prophylactic care to reduce the overall incidence of transplacental fetal infection. GEN

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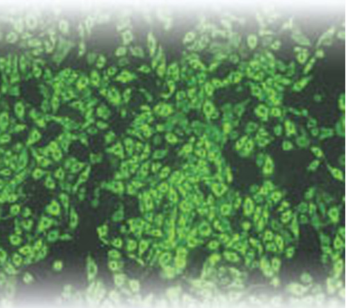

Capabilities:
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- Downstream Filtration and Purification
- Assay Development
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