DEVELOPMENT OF A REGULATORY COMPLIANT VIRUS-LIKE PARTICLE (VLP)-BASED VACCINE TO LASSA HEMORRHAGIC FEVER

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Lassa is an often-fatal hemorrhagic illness named for the town in the Yedseram River valley of Nigeria in which the first described cases occurred in 1969. Parts of Sierra Leone, Guinea, Nigeria, and Liberia are endemic for the etiologic agent, Lassa virus (LASV). The public health impact of LASV in endemic areas is immense: It has been estimated that there are up to 300,000 cases of Lassa per year in West Africa and 5,000 deaths. Because of the high case fatality rate, the ability to spread easily by human-human contact and potential for aerosol release LASV is classified as a Biosafety Level 4 and NIAID Biodefense category A agent, with high bioterrorism potential. Prior LASV vaccine strategies have employed gamma-irradiated LASV, attenuated reassortant arenaviruses, recombinant vaccinia, vesicular stomatitis, yellow fever, and Venezuelan equine encephalitis virus-like replicon particles expressing LASV antigens. Although partial or complete protection was achieved with some vaccine candidates in guinea pig and non-human primate (NHP) models, all approaches tested lacked the safety and regulatory compliance necessary to generate a safe, well tolerated, broadly protective, mass produced, and cost effective vaccine against LASV.

Most of these issues can be addressed by the development of a mammalian cell-derived virus-like particle (VLP)-based Lassa vaccine. To this end we have designed a mammalian expression vector with features well suited for the production of large quantities of VLP in transfected cells. We have employed the human endothelial kidney cell line HEK-293T/17, which is highly transfectable, and expresses recombinant LASV proteins to high levels. The major immunological determinants of LASV are its glycoprotein complex, arising from the proteolytic cleavage of the precursor GPC protein into GP1, GP2, and the associated signal peptide (SSP), as well as the nucleoprotein (NP). Formation and release of LASV virions requires expression of the viral Z matrix protein. LASV Z alone is sufficient to generate VLP, seen as empty particles budding from cells in electron micrographs. Co-expression of Z, GPC, and NP genes in the same cell resulted in the production and release of VLP that contained all three viral proteins. LASV VLP comprised of Z and GPC were also generated. The resulting VLP were biochemically characterized for total protein content, ratios of Z/GPC/NP, presence of host cell ribosomes and rRNA, and stability in a Tris-NaCl-EDTA or Phosphate Buffered Saline formulation at +4C over 4 months. To date, BALB/c mice immunized with LASV VLP comprised of Z and GPC, and Z, GPC, and NP, using a prime+boost schedule (2 boosts, 2-3 weeks apart) with 10 μg of total pseudoparticle protein per mouse, without adjuvants, generated virion-specific endpoint IgG titers. LASV Z+GPC VLP generated mean titer ~8400 on homologous VLP antigen, GP1-specific IgG ~240, and GP2-specific IgG ~320 (mean, n=10). Whereas, LASV Z+GPC+NP VLP generated mean titer ~25600 on homologous VLP antigen, GP1-specific IgG ~4850, GP2 IgG ~4850, and NP IgG ~1600 (mean, n=10).

Currently, we are scheduling studies at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) Biosafety Level 4 (BSL-4) facilities, to test the host immune response and protective efficacy of LASV-specific virus-like particles (VLPs) in the susceptible guinea pig strain 13 model.

Initial results indicate that a LASV VLP-based vaccine candidate is immunogenic, safe, and well tolerated in a murine model. Future studies will focus on establishing parameters that elicit a broad protective response against lethal Lassa challenge in LASV VLP vaccinated animal models.
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