PALADINS: PROTECTIVE APPROACHES LEVERAGING AD-APTIVE AND IN-NATE SYSTEMS

Monoclonal/polyclonal Antibodies Preparation To Investigate Lassa Virus

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LASV has the highest human impact of any of the viral hemorrhagic fevers (with the exception of Dengue Fever) with an estimated several hundred thousand infections annually, resulting in thousands of deaths in Western Africa. The sizeable disease burden, numerous imported cases of LF in non-endemic countries, and the possibility that LASV can be used as an agent of biological warfare make a strong case for vaccine development. LASV titers are detectable in most tissues, with high titers of LASV in blood, lung, spleen, pancreas, lymph nodes, adrenal gland, kidneys, liver and heart when virus is inoculated subcutaneously (Walker et al., 1975; Jahrling et al., 1982). Animals injected intraperitoneally with reassortant. Four animals per dose will be used, with blood sample collection from two animals per dose at each time point in alternating fashion. Blood samples will be obtained through a jugular cannula and plasma isolation at 15,000 × g for 2 min. at 4 °C in plasma separation tubes, absorption systems , extracted on a Tomtec Quadra 96-Model liquid handling system, and dilution, 4-fold in acetonitrile containing 100 ng/ml ritonavir as the internal standard on a Sirocco Protein Precipitation Plate. The analysis will involve liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a PE, Quantitation against calibration curves will be generated by spiking blank guinea pig plasma. Blood samples will be taken on days −7, 7, 14, 21 and 29 postinfection. Animals will be euthanized when non-ambulatory and/or moribund. Surviving animals will be euthanized and terminal blood samples collection on day 30 of the study (day 29 postinfection). There will be serum samples analyses for viremia, LASV-specific antibodies, neutralizing antibodies, and blood chemistry values. Necropsies will be performed on each animal, and the tissues will be analyzed for LASV-specific histopathological and immunohistochemical analysis. Bound antibodies will be detected with guinea pig-specific anti-IgG1 or anti-IgG2 HRP-conjugated secondary antibodies (Immunological Consultants, Inc.). A colorimetric assay using SureBlue TMB (KPL Laboratories) as substrate perform and absorbance values at A450 will be obtained using a microtiter plate reader.
Analysis of LASV-Specific Antibody Production, Analysis of Viremia, Plaque-Reduction Neutralization Test (PRNT), Blood Chemistry Analysis will be conducted. There will also be pre- and post -infection serum sample collection analysis for glucose, blood urea nitrogen, creatinine, uric acid, calcium albumin total protein,alanine aminotranceferace(ALT), aspartate aminotranceferase (AST), alkaline phosphatase(AP)total bilirubin, gamma glutamyl transferase, and amylase. A Piccolo point- of-care blood chemistry analyzer will be used.

Pathological analysis of tissues, histological processing, immunohitochemistry using commercial mouse monoclonal antibody(immunoperoxidase kit), deparaffnization & peroxidase blocking and counter stain will also performed.