

**SOLUBLE AND MEMBRANE ANCHORED
FORMS OF LASSA VIRUS SUBUNIT
PROTEINS**

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 60/922,732, filed Apr. 10, 2007, which is herein incorporated by reference in its entirety.

**STATEMENT REGARDING FEDERALLY
SPONSORED RESEARCH OR DEVELOPMENT**

[0002] This invention was made, in part, with support provided by the United States government under Grant No. 1 UC1 AI067188-01 awarded by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. The Government may have certain rights in this invention.

FIELD OF THE INVENTION

[0003] This present invention relates to novel forms of protein subunits from Lassa virus (LASV), to compositions comprising the novel forms of protein subunits from LASV, and methods comprising the same.

BACKGROUND

[0004] Lassa virus (LASV) and several other members of the Arenaviridae are classified as Biosafety Level 4 and NIAID Biodefense Category A agents. The proposed studies will fill a vital biodefense need for rapid multiagent immunodiagnostic assays for arenaviruses, and provide a major advance for public health management of an important family of viral pathogens.

[0005] Lassa fever. The most prevalent arenaviral disease is Lassa, an often-fatal hemorrhagic fever named for the Nigerian town in which the first described cases occurred in 1969 (Buckley and Casals, 1970). Parts of Guinea, Sierra Leone, Nigeria, and Liberia are endemic for the etiologic agent, LASV (Birmingham and Kenyon, 2001). Although detailed surveillance of LASV is hampered by many factors, including the lack of a widely available diagnostic test, it is clear that the public health impact is immense. There are as many as 300,000 cases of Lassa per year in West Africa and 5,000 deaths (see [http site for www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm](http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm)). In some parts of Sierra Leone, 10-16% of all patients admitted to hospitals have Lassa fever. Case fatality rates for Lassa fever are typically 15% to 20%, although in epidemics overall mortality can be as high as 45%. LASV has been associated with severe nosocomial outbreaks involving health care workers and laboratory personnel (Fisher-Hoch et al., 1995). The mortality rate for women in the last month of pregnancy is always high, ~90%, and LASV infection causes high rates of fetal death at all stages of gestation (Walls, 1985). Mortality rates for Lassa appear to be higher in non-Africans, which is of concern because Lassa is the most commonly exported hemorrhagic fever (Haas et al., 2003; Holmes et al., 1990).

[0006] Old and New World arenaviruses. The genome of arenaviruses consists of two segments of single-stranded, ambisense RNA. There are three major structural proteins, including two envelope glycoproteins (GP1 and GP2) and the nucleocapsid protein (NP). The structure of arenavirus GP2 appears to be a class I fusion protein, which is common to envelope glycoproteins of myxoviruses, retroviruses and

filoviruses (Gallagher, DiSimone, and Buchmeier, 2001). When viewed by transmission electron microscopy, the enveloped spherical virions (diameter: 110-130 nm) show grainy particles that are ribosomes acquired from the host cells (Murphy and Whitfield, 1975). Hence the use for the family name of the Latin word "arena," which means "sandy." The arenaviruses are divided into the Old World or lymphocytic choriomeningitis virus (LCMV)/LASV complex and the New World or Tacaribe complex (Bowen, Peters, and Nichol, 1997). There is considerable diversity amongst members of the Arenaviridae (FIG. 1), and even within the same virus species (Bowen et al., 2000). In addition to LASV, other arenaviruses that cause severe illness in humans and are classified as BSL-4 and NIAID category A agents, include the New World arenaviruses Machupo virus (MACV, Bolivian hemorrhagic fever), Junin virus (JUNV, Argentine hemorrhagic fever), Guanarito virus (GUAV, Venezuelan hemorrhagic fever) and Sabia virus (SABV, Brazilian hemorrhagic fever). Arenaviruses are zoonotic; each virus is associated with a specific species of rodent (Bowen, Peters, and Nichol, 1997). The LCMV/LASV complex viruses are associated with Old World rats and mice (family Muridae, subfamily Murinae). Tacaribe complex viruses are generally associated with New World rats and mice (family Muridae, subfamily Sigmodontinae); however, the reservoir of Tacaribe virus itself appears to be a bat (Bowen, Peters, and Nichol, 1996). The reservoir of LASV is the "multimammate rat" of the genus *Mastomys* (Monath et al., 1974). *Mastomys* rats are ubiquitous in sub-Saharan Africa (Demby et al., 2001) and are known to be peridomestic, often living in human homes; however, many questions regarding the taxonomy, geographic distribution and ecobiology of *Mastomys* species are unanswered. As with the natural hosts of other arenaviruses, *Mastomys* show no symptoms of LASV infection, but shed the virus in saliva, urine and feces. Eradication of the widely distributed rodent reservoirs of LASV and other arenaviruses is impractical and ecologically undesirable.

[0007] Arenaviruses are easily transmitted to humans via direct contact with rodent excreta or by contact with or ingestion of excreta-contaminated materials (Bausch et al., 2001; Demby et al., 2001). Infection usually occurs via mucous membranes or skin breaks. In the case of *Mastomys* species, infection may also occur when the animals are caught, prepared as a food source and eaten. Most arenaviruses, including LASV, are readily transmitted between humans, thus making nosocomial infection another matter of great concern. Human-to-human transmission can occur via exposure to blood or body fluids. LASV can also be transmitted to sexual partners of convalescent men via semen up to six weeks post-infection.

[0008] Natural history of Lassa fever. Signs and symptoms of Lassa fever, which occur 1-3 weeks after virus exposure, are highly variable, but typically begin with the insidious onset of fever and other nonspecific symptoms such as headache, generalized weakness, and malaise, followed within days by sore throat, retrosternal pain, conjunctival injection, abdominal pain, and diarrhea. LASV infects endothelial cells, resulting in increased capillary permeability, which can produce diminished effective circulating volume (Peters et al., 1989). Severe cases progress to facial and neck swelling, shock and multiorgan system failure. Frank bleeding, usually mucosal (gums, etc.), occurs in less than a third of cases, but confers a poor prognosis. Neurological problems have also been described, including hearing loss, tremors, and encephal-