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Development of live-attenuated arenavirus vaccines based on codon deoptimization of the viral glycoprotein

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ABSTRACT

Several arenaviruses, chiefly Lassa (LASV) in West Africa, cause hemorrhagic fever (HF) disease in humans and pose important public health problems in their endemic regions. To date, there are no FDA-approved arenavirus vaccines and current anti-arenaviral therapy is limited to the use of ribavirin that has very limited efficacy. In this work we document that a recombinant prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) with a codon deoptimized (CD) surface glycoprotein (GP), rLCMV/CD, exhibited wild type (WT)-like growth properties in cultured cells despite barely detectable GP expression levels in rLCMV/CD-infected cells. Importantly, rLCMV/CD was highly attenuated *in vivo* but able to induce complete protection against a subsequent lethal challenge with rLCMV/WT. Our findings support the feasibility of implementing an arenavirus GP CD-based approach for the development of safe and effective live-attenuated vaccines (LAVs) to combat diseases caused by human pathogenic arenaviruses.

1. Introduction

Several members in the *Arenaviridae* family cause hemorrhagic fever (HF) disease in humans (Buchmeier et al., 2007; McCormick and Fisher-Hoch, 2002). Thus, Lassa (LASV) in West Africa and Junin (JUNV) in the Argentine Pampas, cause Lassa fever (LF) and Argentine HF, respectively, diseases in humans that are associated with high morbidity and significant mortality, and pose an important public health problem in their endemic areas (Borio et al., 2002; Buchmeier et al., 2007; McCormick and Fisher-Hoch, 2002). Moreover, increased travel has resulted in the importation of LF cases into non-endemic metropolitan regions in the USA, Europe and Japan (Buchmeier et al., 2007; Holmes et al., 1990; Isaacson, 2001). Moreover, novel arenaviruses are being discovered every one to three years (Kunz, 2009), including the recent identification of two novel HF-causing arenaviruses: Chapare in Bolivia in 2003 (Delgado et al., 2008) and Lujo in Southern Africa in 2008 (Briese et al., 2009). It should be also noted that mounting evidence indicates that the worldwide-distributed prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) is a neglected human pathogen of clinical relevance (Fischer et al., 2006; Palacios et al., 2008; Schafer et al., 2014). In addition, several arenaviruses pose a credible bioterrorism threat and six of them, including LASV and JUNV are classified as Category A agents by the National Institute of Allergy and Infectious Diseases (NIAID) (Borio et al., 2002; Charrel and de Lamballerie, 2003). Despite the significance of arenaviruses in public health and biodefense readiness, to date there are no vaccines approved by the Food and Drug Administration (FDA) and current anti-arenavirus therapy is limited to the off-labeled use of the broad-spectrum nucleoside analog ribavirin that is only partially effective and requires an early and intravenous administration and can also cause significant side effects (Kilgore et al., 1997; McKee et al., 1988; Snell, 1988).

Epidemiological studies indicate that live-attenuated vaccines (LAV) represent the most feasible approach to control HF arenaviruses within their endemic regions, as LAV induce long-term robust cellular and humoral immune responses following a single immunization (Falzarano and Feldmann, 2013; Fisher-Hoch and McCormick, 2004; Lukashevich, 2012; McCormick and Fisher-Hoch, 2002). The JUNV live-attenuated Candid#1 strain developed from a joint effort between the USA Army Medical Research Institute of Infectious Diseases (USAMRIID) and the Argentine Ministry of Health in the early 1990 s, has been shown to be an effective vaccine against Argentine HF (Enria et al., 2008, 2010; Maiztegui et al., 1998; McKee et al., 1992). However, outside Argentina, Candid#1 remains as an investigational new drug (IND). The reassortant ML29 carrying the L segment from the non-pathogenic Mopeia virus (MOPV) and the S segment

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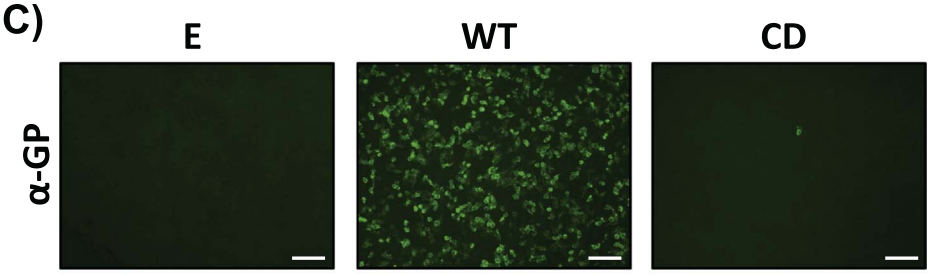
A)

MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFAL
ISFLLLAGRSCGMYGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNAC
SANNSHHYISMGTSGLELTFTNDSIISHNFCNLTSAFNKKTFDHTL
MSIVSSLHLSIRGNSNYKAVSCDFNNGITIQYNLTFSNAQSAQSQC
RTFRGRVLDMFRTAFGGKYMRSGWGWTGSDGKTTWCSQTSYQYLII
QNRTWENHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSS
GVENPGGYCLTKWMILAAELKCFGNTAVAKCNVNHDEEFCDMLRLI
DYNKAALSKFKEDVESALHLFKTTVNSLISDQLLMRNHLRDLMGVP
YCNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIEQEAD
NMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYLVSIFLHLVKIPT
HRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKRR

B)

LCMV GP CD

Mutations (nucleotides)	% CD (nucleotides)	Mutations (amino acids)	% CD (amino acids)
431/1494	28.8	357/498	71.7



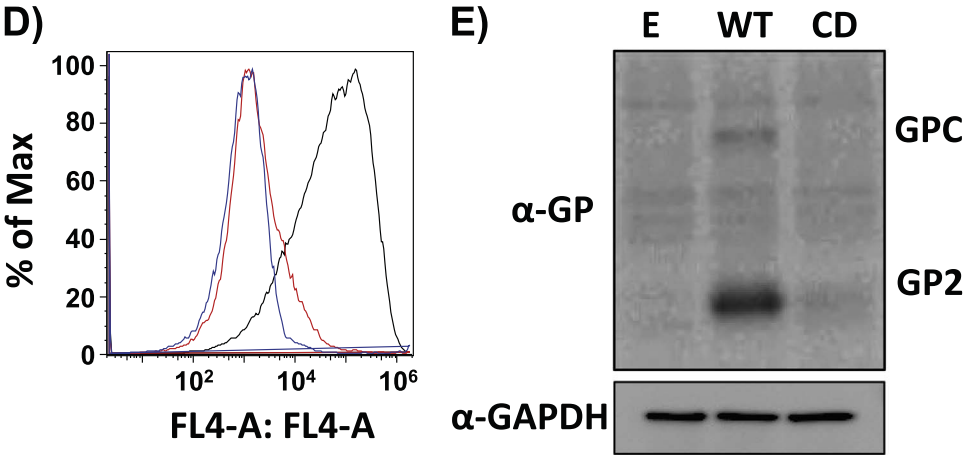


Fig. 1. CD reduces LCMV GP expression levels. A) Amino acid sequence of LCMV GP CD. CD LCMV GP amino acid residues are indicated in red. Methionine (M) and tryptophan (T) residues in LCMV GP, as well as amino acids already associated with deoptimized codons are indicated in black. Underlined (solid) amino acids represent the stable signal peptide (SSP). No underlined region represents GP1. Underlined (dotted) amino acids represent GP2. Box represents the GP transmembrane domain. Amino acids highlighted in gray represent the GP cytoplasmic tail. B) Mutations in LCMV GP CD. Number of nucleotide mutations and the percentage (%) of CD amino acids are indicated. C-E) LCMV GP CD expression levels in transfected cells. Human 293 T cells were transiently transfected with pCAGGS expression plasmids encoding LCMV GP WT or GP CD and at 48 h p.t. GP expression was assessed by IFA (C), FACS (D) and WB (E) using the LCMV GP mouse monoclonal antibody 83.6 (GP2). Empty (E) plasmid was included as negative control (C-D). IFA, FACS and WB results correspond to representative of three independent transfection experiments. (C) Scale bar=100 μm. (D) Blue line: mock-transfected cells. Black line: cells transfected with LCMV GP WT. Red line: cells transfected with LCMV GP CD. (E) GAPDH expression levels were used as loading controls.

from LASV has been shown to be safe, immunogenic and capable of provide protection in guinea pig and non-human primate models of LF (Carrion et al., 2007; Lukashevich et al., 2005, 2008). Differences in polymerase activity between LASV and MOPV likely contributed to ML29 attenuation but the mechanisms of attenuation of ML29 remain unknown, which raises concerns about whether the acquisition of additional mutations by the L polymerase of ML29 could result in increased virulence. Likewise, there are concerns that potential reas-