Virology 512 (2017) 161–171

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Oxidation-sensitive polymersomes as vaccine nanocarriers enhance humoral responses against Lassa virus envelope glycoprotein

Clara Galan-Navarro[a,b], Marcela Rincon-Restrepo[b], Gert Zimmer[c], Erica Ollmann Saphire[d,e], Jeffrey A. Hubbell[b,f], Sachiko Hirosue[b,*], Melody A. Swartz[b,f,*], Stefan Kunz[a,*]

[a] Institute of Microbiology, Lausanne University Hospital. Lausanne, Switzerland
[b] Laboratory of Lymphatic and Cancer Bioengineering, Institute of Bioengineering, École Polytechnique Féderal de Lausanne (EPFL), 1015 Lausanne, Switzerland
[c] Division of Virology, Institute of Virology and Immunology, 3147 Mittelhäusern, Switzerland
[d] Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA, United States
[e] Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA, United States
[f] Institute for Molecular Engineering and Ben May Department of Cancer Research, University of Chicago, IL, United States

ARTICLE INFO

Keywords:
Polymersomes
Nanocarrier
Lassa virus
Anti-viral antibodies
Subunit vaccine

ABSTRACT

Lassa virus (LASV) causes severe hemorrhagic fever with high mortality, yet no vaccine currently exists. Antibodies targeting viral attachment proteins are crucial for protection against many viral infections. However, the envelope glycoprotein (GP)−1 of LASV elicits weak antibody responses due to extensive glycan shielding. Here, we explored a novel vaccine strategy to enhance humoral immunity against LASV GP1. Using structural information, we designed a recombinant GP1 immunogen, and then encapsulated it into oxidation-sensitive polymersomes (PS) as nanocarriers that promote intracellular MHCII loading. Mice immunized with adjuvanted PS (LASV GP1) showed superior humoral responses than free LASV GP1, including antibodies with higher binding affinity to virion GP1, increased levels of polyfunctional anti-viral CD4 T cells, and IgG-secreting B cells. PS (LASV GP1) elicited a more diverse epitope repertoire of anti-viral IgG. Together, these data demonstrate the potential of our nanocarrier vaccine platform for generating virus-specific antibodies against weakly immunogenic viral antigens.

1. Introduction

Lassa virus (LASV) is an Old World arenavirus that causes a severe viral hemorrhagic fever with high mortality in humans (Geisbert and Jahrling, 2004; McCormick and Fisher-Hoch, 2002) and is currently considered one of the most important emerging pathogens by WHO (Sweileh, 2017). Endemic in Western Africa from Senegal to Cameroon, LASV causes several hundred thousand infections per year with thousands of deaths. In nature, LASV is carried by persistent infection of reservoir rodent host of *Mastomys* species, semi-domestic rodents that invade human dwellings (McCormick and Fisher-Hoch, 2002). Reservoir-to-human transmission represents a major route of human infection (Andersen et al., 2015) and human-to-human transmission has been reported in nosocomial outbreaks (Fisher-Hoch et al., 1995). Due to its transmissibility via aerosol (Stephenson et al., 1984) and high lethality, LASV is further considered a category A select agent by the Centers of Disease Control (Borio et al., 2002). There is currently no licensed vaccine available and the standard of care is limited to supportive measures and the use of ribavirin, which reduces mortality when delivered early in infection (McCormick et al., 1986). Severe Lassa fever in humans is characterized by extensive viral replication and spread, resulting in high viremia and progressive signs and symptoms of shock. Viral load early in disease is predictive for fatal disease outcome, indicating competition between viral spread and replication and the patient's immune system (McCormick et al., 1987). Control of primary LASV infection in survivors seems mainly mediated by the anti-viral CD8 T-cell response, whereas neutralizing antibodies appear late during convalescence and are generally of low titers (Prescott et al., 2017).

Antibodies represent a major immunological correlate of protection in many successful vaccines. These protective antibodies control the initial burst of viral replication by neutralizing free virus, thus limiting viral spread. The consequent reduction in viral load provides the host's immune system a window of opportunity to develop a timely adaptive response capable of controlling the pathogen (Burton et al., 2012). Studies in numerous viral systems demonstrated that antibodies targeting viral attachment proteins are of particular importance, as they can neutralize free virus by preventing host cell attachment and entry

* Correspondence to: Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne CH-1011, Switzerland.
E-mail addresses: sach@alum.mit.edu (S. Hirosue), melodyswartz@uchicago.edu (M.A. Swartz), Stefan.Kunz@chuv.ch (S. Kunz).

http://dx.doi.org/10.1016/j.virol.2017.09.013
Received 14 July 2017; Received in revised form 12 September 2017; Accepted 14 September 2017

(Burton et al., 2001; Karlsson Hedestam et al., 2008; Parren and Burton, 2001). Recently, recombinant monoclonal antibodies (mAb) targeting viral attachment proteins showed promise for protection in prophylactic, post-exposure, and therapeutic settings, as illustrated in groundbreaking studies with recombinant antibody cocktails against Ebola virus (Zeitlin et al., 2016). Specific antibodies to viral surface proteins are further of great importance in viral serology diagnostics and epidemiology, in particular in the context of newly emerging or re-emerging pathogens.

Initial trials with killed LASV vaccines failed to elicit protective immunity (McCormick et al., 1992), and the development of a safe and efficacious LASV subunit vaccine capable of inducing robust antibodies titers remains an unsolved problem (Olschlager and Flatz, 2013). A major challenge is the low immunogenicity of the LASV envelope GP1 which decorates the virion spikes, is involved in host cell attachment, and represents the main target for protective antibodies (Borrow and Oldstone, 1992; Robinson et al., 2016; Sanchez et al., 1989). A recent elegant study in a mouse model demonstrated that the inherently low immunogenicity of LASV GP1 is a consequence of extensive epitope shielding by N-linked glycans (Sommerstein et al., 2015). This is reminiscent of the surface GPs of other major human pathogens, including human immunodeficiency virus (HIV) − 1 and hepatitis C virus (HCV) (Burton and Mascola, 2015; Burton et al., 2012).

To enhance immunogenicity of subunit vaccines, a range of antigen nanocarriers have been developed, including polymer-based nanoparticles, liposomes, polymersomes, dendrimers, cyclodextrin-containing polymers, carbon nanotubes, and gold nanoparticles (Webster et al., 2013). We recently developed a novel watery-core polymersome (PS) nanocarrier system designed to efficiently deliver antigen to lymph nodes, enhance uptake by dendritic cells (DCs), and promote antigen presentation (Scott et al., 2012; Stano et al., 2013). Polymersomes are stable vesicles composed of self-assembling oxidation-sensitive block co-polymers, in particular hydrophobic polypropylene sulfide (PPS) in combination with hydrophilic polyethylene glycol (PEG) (Scott et al., 2012). Hydrophilic molecules such as soluble recombinant protein antigens and some adjuvants can be incorporated into the watery core of PEG-*bl*-PPS PS, whereas hydrophobic molecules can associate with the leaflet of the membrane bilayer. Previous studies demonstrated that incorporation of model protein antigens into the aqueous core of PS enhances antigen presentation by dendritic cells in vitro (Scott et al., 2012). *In vivo*, PS induced robust T cell immunity against protein antigens with enhanced frequencies of antigen-specific CD4 T cells (Stano et al., 2013).

In our present study, we evaluated a new vaccine, consisting of our PS nanocarrier platform in combination with a newly developed immunogen, for its ability to enhance the humoral immune response to the weakly immunogenic LASV GP1 in a mouse model.

2. Results

2.1. Immunogen design, production, and characterization

The LASV glycoprotein precursor GPC is initially synthesized as a single polypeptide that is proteolytically processed into the peripheral GP1 and the membrane-associated GP2 (Fig. 1A). The N-terminal GP1 decorates the tips of the trimeric virion spike and is implicated in receptor binding (Li et al., 2016), whereas the transmembrane GP2 resembles fusion-active class I envelope proteins of other viruses (Igonet et al., 2011). LASV GP1 represents an independent fold with a globular structure (Cohen-Dvashi et al., 2015). Based on available structural information, we engineered a stable, soluble LASV GP1 fragment comprised of amino acids 92–256, spanning the putative receptor binding sites (Cohen-Dvashi et al., 2015; Illick et al., 2008) and known neutralizing epitopes (Robinson et al., 2016) (Fig. 1A). Removal of the 33 N-terminal amino acids of GP1 markedly enhanced expression without affecting the overall fold of the protein (Bowden et al., 2009). To ensure high expression levels and proper folding, recombinant LASV GP1 was expressed in mammalian cells as a C-terminal fusion protein with the Fc moiety of human IgG1 (Radoshitzky et al., 2007). To allow efficient removal of the human Fc part after purification, an enterokinase (EK) cleavage site was inserted following the C-terminus of LASV GP1, resulting in the construct LASVGP1-EK-Fc (Fig. 1A). Western blot of the recombinant protein expressed in HEK293H cells revealed the expected apparent molecular masses of the GP1-Fc fusion protein that formed the expected dimer (Fig. 1B)

Large-scale protein expression was carried out in high-density suspension cultures under serum-free conditions, followed by the purification strategy outlined in Fig. 1C. Briefly, conditioned cell culture supernatant was subjected to protein A affinity chromatography, followed by EK cleavage performed on the column, allowing selective elution of LASV GP1 under physiological conditions. Eluted LASV GP1 was further purified by ion exchange chromatography followed by gel filtration, resulting in > 98% pure protein with the expected apparent molecular mass (Fig. 1D). Our construct design resulted in formation of an intermolecular disulfide bond via C230. Accordingly, SDS-PAGE under non-reducing conditions revealed that LASV GP1 formed a dimer (Fig. 1E). To further confirm the expected molecular mass and dimerization of LASV GP1, we combined size-exclusion chromatography with multi angle light scattering (SEC-MALS) (Fig. S1). From the detected molecular mass of the LASV GP1 dimer of 56.1 kDa, only 30.5 kDa corresponded to the predicted polypeptide, indicating extensive post-translational modifications, mainly N-glycosylation, in line with previous studies (Branco et al., 2010; Illick et al., 2008). Examination of the glycosylation pattern of LASV GP1 revealed the presence of high mannose N-glycans (Fig. S2), similar to GP1 derived from authentic virus (Goncalves et al., 2013) and recombinant full-length GP1 (Branco et al., 2010). To assess the correct conformation of our recombinant LASV GP1, we tested binding of a panel of previously described conformation-sensitive anti-GP1 mAbs (Robinson et al., 2016) in ELISA. As shown in Fig. S3, all mAb tested recognized our LASV GP1. Together, the data suggest that our LASV GP1 retains at least part of its native conformation and displays a glycosylation pattern similar to GP1 from authentic virus, making it a suitable immunogen for our studies.

2.2. Preparation of polymersomes loaded with LASV GP1 antigen

PEG-*bl*-PPS PS (Fig. 2A) were synthesized as outlined in Fig. 2B and described in Materials and Methods (Scott et al., 2012; Stano et al., 2013). To monitor efficiency of immunogen encapsulation, recombinant purified LASV GP1 was fluorescence labeled with the fluorophore AF594. Considering the small molecule nature of the fluorophore, we did not expect a significant impact on the physicochemical nature of our recombinant immunogen. After 48 h incubation at 4 °C, PS were extruded and purified by gel filtration chromatography. A significant proportion of LASV GP1 co-eluted with the PS fraction, indicating efficient antigen loading (Fig. 2C). To validate the integrity of the encapsulated antigen, loaded PS were lysed with 0.5% Triton-X 100 and analyzed by SDS-PAGE. The antigen recovered from PS migrated similar to the input, indicating conservation of the dimeric from during the encapsulation process (Fig. 2D). Remaining free LASV GP1 was removed by size exclusion chromatography. Nanoparticles in the 100 nm range ensure efficient lymphatic transport and delivery to antigen-presenting cells in lymph nodes upon intradermal or subcutaneous immunization (Stano et al., 2013). The resulting PS (LASV GP1) displayed an average size range of 163 ± 7 nm, with a polydispersity index of 0.1 ± 0.05 (Fig. 2E), which was suitable for our immunization studies.

2.3. Delivery of LASV GP1 via PS enhances the quality of the antibody response

To evaluate the capacity of PS to induce an anti-LASV GP1 antibody