Virology 456-457 (2014) 55–69

Contents lists available at ScienceDirect

Virology

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

Multi-parameter exploration of HIV-1 virus-like particles as neutralizing antibody immunogens in guinea pigs, rabbits and macaques

Tommy Tong [a], Ema T. Crooks [a], Keiko Osawa [a], James E. Robinson [b], Mary Barnes [c], Cristian Apetrei [c,1], James M. Binley [a,*]

[a] Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, CA 92121, USA
[b] Tulane University, 1430 Tulane Avenue, New Orleans, LA 70112, USA
[c] Tulane National Primate Research Center, 18703 Three Rivers Road, Covington, LA 70433, USA

ARTICLE INFO

Article history:
Received 28 January 2014
Returned to author for revisions
26 February 2014
Accepted 13 March 2014
Available online 29 March 2014

Keywords:
HIV
Vaccine
Antibody
Neutralization
VLPs
gp120
gp41
Env

ABSTRACT

Virus-like particles (VLPs) offer a platform to test the hypothesis that, since antibody binding to native envelope glycoprotein (Env) trimers results in HIV-1 neutralization, that native Env trimers presented in membranes may be useful for inducing neutralizing antibodies (nAbs) in a vaccine setting. So far, VLPs have not fulfilled this potential. Here, using a "shotgun" approach, we evaluated a wide cross-section of variables in a series of VLP immunizations. We identified 3 tentative leads. First, that VLP doses may not have been sufficient for optimal nAb induction. Second, that dampening the antigenicity of non-functional Env (for example uncleaved gp160) using either protease digests or IgG masking may be useful. Third, that guinea pig sera preferentially target non-conserved epitopes and exhibit relatively high background activity, suggesting that rabbits may be preferable as small animal vaccine models. Recent immunogenicity studies in rabbits appear to bear out all 3 of these leads.

Introduction

Designing a vaccine for human immunodeficiency virus type 1 (HIV-1) remains a major challenge of modern biomedical research (Schiffner et al., 2013). Broadly neutralizing antibodies (bnAbs) are expected to be a key component of the protective immunity imparted by an effective HIV-1 vaccine (Mascola and Montefiori, 2010). However, current vaccine candidates usually fail to elicit effective tier 2 neutralization even against the vaccine strain – and when they do so, it is usually elicited inconsistently in only a few immunized animals and largely fails to cross-neutralize other tier 2 strains (Klasse et al., 2012; Mascola and Montefiori, 2010; McCoy and Weiss, 2013; Schiffner et al., 2013; van Gils and Sanders, 2013).

HIV-1 neutralization occurs when antibodies bind to native, functional Env spikes arrayed on viral membranes, thereby interfering in their engagement with cellular receptors (Mascola and Montefiori, 2010). Functional Env spikes consist of trimers of gp120/gp41 heterodimers in which the surface gp120 subunit mediates receptor binding and the membrane-anchoring gp41 subunit mediates fusion. These two subunits derive from a gp160 precursor that is glycosylated co-translationally (Earl et al., 1990), and later processed by a furin-like enzyme to form native gp120/gp41 trimers that are incorporated into nascent particles.

Mature Env spikes are compact and heavily glycosylated, thus allowing them to evade antibody binding and neutralization (Kwong and Mascola, 2012; Moore et al., 2006; van Gils and Sanders, 2013). Env can exist in various other forms, including the uncleaved (UNC) gp160 precursor, soluble monomeric gp120 and gp41 stumps, all of which are relatively accessible to antibody binding (Tong et al., 2012, 2013). It is perhaps not altogether surprising then that vaccines based on these and other relatively "accessible" forms of Env elicit antibodies that are largely unable to penetrate the compact native trimer's natural defenses (Klasse et al., 2012; Mascola and Montefiori, 2010; McCoy and Weiss, 2013; Schiffner et al., 2013). Conversely, the native Env trimer itself, presented in its natural context on lipid membranes might have the requisite stringency as an immunogen to consistently

* Corresponding author. Tel.: +1 858 597 3842; fax: +1 858 455 3804.
E-mail address: jbinley@tpims.org (J.M. Binley).
[1] Present address: University of Pittsburg Center for Vaccine Research, 9044 Biomedical Science Tower 3, 3501 Fifth Avenue, Pittsburgh, PA 15261, USA.

http://dx.doi.org/10.1016/j.virol.2014.03.015
0042-6822/

induce antibodies capable of penetrating its own highly sophisticated defenses – if optimal immunization conditions can be identified (Emini and Koff, 2004).

A natural lipid membrane context may be important for a fully native Env trimer conformation. Considering that all currently licensed vaccines against infectious diseases and several others under development are particle-based, many of which incorporate lipid membranes, this general approach has a strong track record (Garrone et al., 2011; Kanekiyo et al., 2013; Kulkarni et al., 2012; Kushnir et al., 2012; Roldao et al., 2010). Particle vaccines presenting native HIV-1 Env spikes have so far been explored in the form of live inactivated viruses, VLPs, liposomes and virosomes (Bomsel et al., 2011; Buonaguro et al., 2005; Chen et al., 2005; Crooks et al., 2007; Dennison et al., 2011; Doan et al., 2005; Evans et al., 2005; Grovit-Ferbas et al., 2000; Grundner et al., 2002; Hammonds et al., 2003, 2005, 2007; Hicar et al., 2010; Kamdem Toukam et al., 2012; Lifson et al., 2004; McBurney et al., 2007; McKenna et al., 2004, 2003; Montero et al., 2012; Pastori et al., 2012; Poon et al., 2005; Visciano et al., 2011; Vzorov et al., 1999; Yang et al., 2012; Zhou et al., 2011). However, none have yet demonstrated a great potential to elicit tier 2 nAbs. One explanation for this lack of progress may be that the native Env trimer's compact nature renders it an inherently poor immunogen (see Fig. 4 in Tong et al. (2013)). It therefore remains possible that higher native Env trimer doses and/or the use of powerful dose-sparing adjuvants can address problem (Li et al., 2006; VanCott et al., 1997).

Another problem with particle-based vaccines is that they carry non-functional Env on their surfaces, principally uncleaved (UNC) gp160 and gp41 stumps that are relatively accessible to binding by non-neutralizing antibodies and may therefore interfere with the development of neutralizing responses to the native Env trimer (Agrawal et al., 2011; Crooks et al., 2007; Hicar et al., 2010; Joyner et al., 2011; Leaman et al., 2010; Moore et al., 2006; Nyambi et al., 1998; Tong et al., 2012, 2013). One solution may be to extract and purify soluble native Env trimers from membranes. However, this is hampered by the instability of the isolated product and its tendency to aggregate. Another solution may be to mask non-functional Env using IgG. This idea has some precedents (Denisova et al., 1996; Keller and Arora, 1999; Liao et al., 2004; Visciano et al., 2008). Furthermore, IgG complexing may facilitate VLP adsorption to follicular dendritic cells (Abdel-Motal et al., 2010; Forthal et al., 2007; van Montfort et al., 2007) and antigen processing (Hioe et al., 2009). A more recent option is to treat VLPs with proteases to selectively digest and thereby remove non-functional Env, leaving native Env trimers intact (Crooks et al., 2011; Tong et al., 2012).

A growing number of broadly neutralizing monoclonal antibodies recovered from infected donors to date are considered to be blueprints for vaccine discovery. Their sophisticated features, however, raise questions about whether model species used for vaccine testing have sufficient germline immunoglobulin repertoire complexity to develop similar nAbs (Pinheiro et al., 2011). This uncertainty calls for the continued testing of nAb vaccine candidates in multiple animal models.

The goal in this study was to identify leads important for eliciting tier 2 nAbs by VLP immunogens. Investigating all of the above-mentioned variables would require very large animal numbers and high costs. Here, using limited resources, and building on our previous VLP immunogenicity study in guinea pigs (Crooks et al., 2007), as outlined in Fig. 1, we examined a wide cross-section of variables in a series of immunization studies in guinea pigs, macaques and rabbits to try to partition factors that might associate with nAb development. Our findings suggest that (i) higher VLP doses, (ii) dampening the antigenicity of non-functional Env from VLP surfaces and (iii) the preferential use of rabbits over guinea pigs as a small animal may be important. All three of these leads appear to be validated in our more recent VLP immunogenicity studies in rabbits.

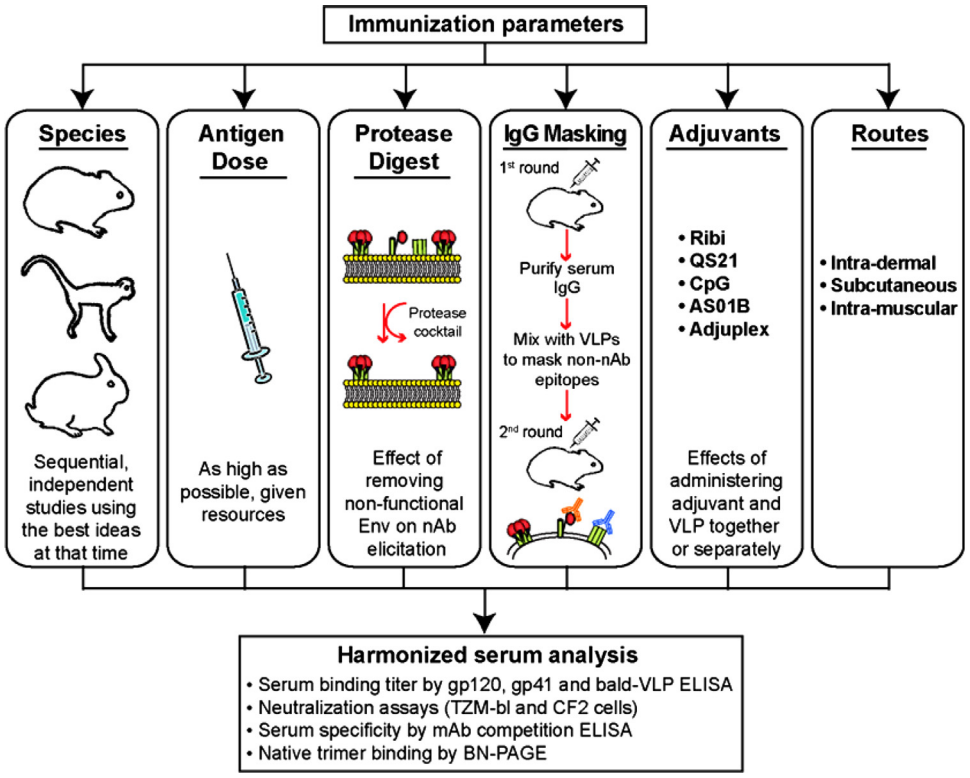

Fig. 1. Overview of immunization parameters. Various immunization parameters, as indicated, were assessed in a shotgun approach to try to determine those that might partition with nAb development. A consistent algorithm to analyze sera was used to enhance our ability to compare strategies between different immunization groups and species.