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Research paper

Label free checkerboard assay to determine overlapping epitopes of Ebola virus VP-40 antibodies using surface plasmon resonance

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ABSTRACT

Immunoassay formats, in which antibodies provide sensitivity and specificity, are often utilized to provide rapid and simple diagnostic tests. Surface plasmon resonance is frequently used to evaluate the suitability of antibodies by determining binding kinetics to agents or surrogate antigens. We used SPR to evaluate a number of commercial monoclonal antibodies as well as single domain antibodies produced in-house. All the antibodies targeted the Ebola virus viral protein 40 (VP40). We determined the ability of each antibody to bind to immobilized VP40, and ensured they did not bind Ebola glycoprotein or the nucleoprotein. A subset of the monoclonal antibodies was immobilized to characterize antigen capture in solution. It can be advantageous to utilize antibodies that recognize distinct epitopes when choosing reagents for detection and diagnostic assays. We determined the uniqueness of the epitope recognized by the anti-VP40 antibodies using a checkerboard format that exploits the 6 × 6 array of interactions monitored by the Bio-Rad ProteOn XPR36 SPR instrument. The results demonstrate the utility of surface plasmon resonance to characterize monoclonal and recombinant antibodies. Additionally, the analysis presented here enabled the identification of pairs of anti-VP40 antibodies which could potentially be utilized in sandwich type immunoassays for the detection of Ebola virus.

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1. Introduction

The Ebola virus (EBOV, formerly the Zaire ebolavirus), is a member of the *Filoviridae* family of viruses (Beeching et al., 2014). As highlighted by the 2014–2015 outbreak in West Africa, filoviruses are human health threats via new emergences, and also pose a threat through importation by infected travelers and health care workers. Antibodies have proven the basis for the development of simple, inexpensive, rapid and sensitive diagnostic tools. In these immunoassay formats, antibodies provide for the assay's sensitivity and specificity. Antibodies have also been shown to be valuable as potential therapeutics, such as the experimental Zmapp cocktail (Qiu et al., 2013; Qiu et al., 2014).

Ebola viruses possess a negative-sense RNA genome (~19 kb) that codes for seven proteins, which also serve as potential immunogens. The nucleoprotein (NP) encapsulates the RNA genome; along with the NP, viral protein 35 (VP35), viral protein 30 (VP30), and the RNA-dependent RNA polymerase (L) are essential for viral replication and transcription. The matrix protein, viral protein 40 (VP40), is critical for virion assembly and budding, and viral protein 24 (VP24) is involved in the formation of nucleocapsid. The viral surface glycoprotein (GP) is involved in viral attachment and entry. GP is present in several different forms with both membrane bound and secreted versions (Sanchez et al., 1996).

Anti-EBOV antibodies have been developed for research, diagnostic, and therapeutic applications including, but not limited to: monoclonal antibodies (mAbs) specific for several forms of GP (Qiu et al., 2011; Shahhosseini et al., 2007; Wilson et al., 2000), NP (Changula et al., 2013) and VP40 (Lucht et al., 2003) as well as recombinantly-derived antibodies (Wang et al., 2015) and antibody binding domains (Anderson et al., 2016; Goodchild et al., 2011; Maruyama et al., 1999; Sherwood and Hayhurst, 2013). Further studies have characterized some of these antibodies in terms of epitope and affinity; for example, the epitopes and binding kinetics of the monoclonal antibodies that make up the ZMapp therapeutic cocktail have been characterized (Audet et al., 2014; Davidson et al., 2015; Murin et al., 2014).

Single domain antibodies (sdAb), are recombinant binding domains derived from the heavy chain only antibodies found in camelids and sharks (Dooley et al., 2003; Ghahroudi et al., 1997; Greenberg et al., 1995; Hamers-Casterman et al., 1993; Nuttall et al., 2004). SdAb are about 10-times smaller than traditional antibodies, comprising the smallest naturally occurring antigen binding domains, but still retain the excellent binding ability and exquisite specificity that are hallmarks of mAbs. Because sdAb are comprised of only one domain, most recover their 3-dimensional structure and binding ability after heat denaturation.

Abbreviations: CRP, Critical Reagents Program; GST, Glutathione S-transferase; GP, Glycoprotein; mAbs, Monoclonal antibodies; NP, Nucleoprotein; sdAb, Single domain antibodies; SPR, Surface plasmon resonance; VP40, Viral protein 40.

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Other advantages include the ability of sdAb to be rationally selected, engineered to improve their properties and tailored to specific applications, and produced in mass quantity by standard recombinant technology (de Marco, 2011; Eyer and Hruska, 2012; Muyldermans, 2013; Wesolowski et al., 2009).

Here, we utilized surface plasmon resonance (SPR) to evaluate a small panel of anti-EBOV antibodies, including both mAbs and newly identified sdAb, specific for VP40. We first determined binding kinetics of each antibody (mAb and sdAb) to immobilized antigen (recombinantly-produced VP40), as well as their ability to bind killed virus. Next, a subset of the monoclonal antibodies were immobilized and evaluated for their ability to capture antigen. This enabled us to rapidly evaluate the ability of the antibodies to function as both reporter and capture reagents. Finally, taking advantage of the ProteOn XPR36's ability to monitor a 6 × 6 array and flow samples in both the horizontal and vertical orientation, we determined the uniqueness of the epitope recognized by the anti-VP40 antibodies, by assessing if the antibodies competed for binding to the VP40 antigen.

There are currently a number of anti-EBOV antibodies and recombinant viral proteins that are commercially available with new products continually coming onto the market. In addition, sdAb specific for several EBOV proteins have been previously identified (Goodchild et al., 2011; Sherwood and Hayhurst, 2013). The current work uses SPR to provide a comparison of both mAb and sdAb reagents. This type of evaluation may provide insights into the most effective reagents and their order of application for development of optimal immunoassays.

2. Materials and methods

2.1. Reagents

Monoclonal antibody reagents, recombinantly produced proteins (VP40, GP, and NP), and killed EBOV were from several commercial vendors as well as from the Department of Defense Critical Reagents Program (CRP) and BEI Resources. All antibody and antigen reagents are detailed in Table 1. The reagents 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysulfosuccinimide (sulfo-NHS) were purchased from Thermo-Fisher. All cloning enzymes were from New England Biolabs. Other chemicals were from VWR or Sigma unless otherwise specified.

2.2. Expression and purification of GST-tagged VP-40

The plasmid encoding glutathione S-transferase (GST)-tagged VP40 (VP40-GST) was a kind gift from Dr. Thomas Hoenen. The construction of the bacterial pGEX-6P-1 expression plasmid was described previously (Hoenen et al., 2010). *E. coli* BL-21 (DE3) were transformed with the plasmid DNA. Six liters of Luria Bertani (LB) broth containing 100 μg/mL of ampicillin were grown to an O.D.600 of ~0.7 and induced with 0.5 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight at 17 °C for approximately 16–17 h. Cells were collected by centrifugation (7000 rpm for 10 min at 4 °C) and lysed in 100 mL of lysis buffer (1× phosphate buffered saline pH 7.4, 5% glycerol, 30 mg lysozyme, 50 U of DNase I). The lysate was sonicated for 60 s and clarified by centrifugation at 20,500 ×g for 30 min at 4 °C. The supernatant was loaded onto a Glutathione Sepharose Fast Flow column (G.E. Healthcare) equilibrated with 1× PBS pH 7.4. The column was washed with 1× PBS pH 7.4 buffer and the protein was eluted with 1× PBS pH 7.4 containing 50 mM glutathione. The eluate was dialyzed against 20 mM Bicine pH 9.8, 100 mM NaCl, 10 mM DTT. The protein was judged to be >95% pure based upon SDS-PAGE gels. The protein was concentrated and stored in 10 mM Bicine pH 9.8, 50 mM NaCl, 5 mM DTT, and 50% glycerol at −20 °C.

2.3. Production of single domain antibodies

Single domain antibodies were derived from a llama which had been immunized with killed virus following a similar protocol and procedure as described previously (Liu et al., 2013; Walper et al., 2014). Llama immunizations were through Triple J farms. Around 300 mL whole blood was drawn 2 weeks after the final immunization. Polyclonal antibody was purified from the plasma; the variable regions of the heavy chain antibody (VHH) were amplified after purifying RNA from the white blood cells and using reverse transcriptase to generate cDNA. Amplified VHH was digested and ligated into the pECAN21 phage display vector (Goldman et al., 2006) for generation of a phage-display sdAb library.

The phage-displayed library was panned using a sandwich method; selection protocols were essentially as described previously (Walper et al., 2014). First, anti-GST mAb (Thermo Scientific) was adsorbed to wells of a Nunc Maxisorp 96-well plate. Next the GST-tagged VP40 was added to wells blocked with 4% non-fat powdered milk. Finally, phage were added to the wells and incubated for ~1–2 h. Wells were washed extensively first with PBST (PBS containing 0.05% Tween 20) followed by washes with PBS. Phage were eluted with 100 mM triethyl amine and neutralized with an equal volume of 1 M Tris, pH 8. Binding phage were identified using monoclonal phage ELISA and unique sdAb identified through sequencing. Representative sdAb were then mobilized from the phage-display vector through *Nco* I-*Not* I to the commercial pET22b for expression.

Proteins were produced as previously described (Goldman et al., 2014). Briefly, plasmids were transformed into Rosetta (DE3) for expression. Overnight cultures (50 mL in Terrific Broth [TB] grown at 25 °C) were used to inoculate 0.5 L of TB which was grown for 3 h at

Table 1
Monoclonal antibodies and antigens.

Antibody/antigen	Source/notes	Catalog number
M82956	Fitzgerald; anti-EBOV VP40	10-2351
M82957	Fitzgerald; anti-EBOV VP40	10-2352
BBI 117	BBI detection; anti-EBOV	BBI 117
BBI 118	BBI detection; anti-EBOV	BBI 118
BBI 119	BBI detection; anti-EBOV	BBI 119
BBI 120	BBI detection; anti-EBOV	BBI 120
BBI 121	BBI detection; anti-EBOV	BBI 121
AB-EB-MAB1	Critical Reagents Program (CRP); anti-EBOV	AB-EB-MAB1
AB-EB-MAB2	Critical Reagents Program (CRP); anti-EBOV	AB-EB-MAB2
Killed virus	Critical Reagents Program (CRP); Inactive Supernatant, Zaire Ebolavirus Mayinga*	ANG-EBOZMSU
Killed virus	BEI Resources, NIAID, NIH: Zaire Ebolavirus Mayinga**	NR-31807
Glycoprotein (GP)	IBT Bioservices; minus transmembrane domain (GPdTM), Sf9 insect cells, PBS	0501-016
VP40	Sino Biological Inc.; Met1-Lys362 N-terminal His-MBP, E. coil, Tris	40446-V10E
VP40	IBT Bioservices, tag-free, *E. coli*, PBS	0501-020
Nucleoprotein (NP)	Sino Biological Inc.; His630-Gln739., *E. coli*, Tris	40443-V07E1

Name, source, and catalog number of mAbs and antigens.
* Gamma-Irradiated; 9 parts "buffer"(EMEM containing 1% glutamine, 5% heat inactivated fetal bovine serum and 1% antibiotics) 1 part Beta-propiolactone (BPL); titer 1.53E + 07.
** Gamma-Irradiated, Borate with 1% Triton X-100.