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Technical note

Plant-based production of two chimeric monoclonal IgG antibodies directed against immunodominant epitopes of *Vibrio cholerae* lipopolysaccharide

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ABSTRACT

We have produced and characterized two chimeric human IgG_1 monoclonal antibodies that bind different immunodominant epitopes on *Vibrio cholerae* lipopolysaccharide (LPS). MAb 2D6 IgG_1 recognizes Ogawa O-polysaccharide antigen, while mAb ZAC-3 IgG_1 recognizes core/lipid A moiety of Ogawa and Inaba LPS. Both antibodies were expressed using a *Nicotiana benthamiana*-based rapid antibody-manufacturing platform (RAMP) and evaluated in vitro for activities associated with immunity to *V. cholerae*, including vibriocidal activity, bacterial agglutination and motility arrest.

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1. Introduction

Vibrio cholerae is the causative agent of cholera, an acute diarrheal disease that remains endemic in many parts of the world. Seven cholera pandemics have been recorded since 1817, with the 7th pandemic wave beginning in 1961 and continuing today. In addition to seasonal outbreaks in areas where *V. cholerae* is endemic, natural disasters such as the 2010 earthquake in Haiti highlight the continued potential for *V. cholerae* to cause mass casualties in regions that were previously cholera-free (Katz et al., 2013).

V. cholerae can be differentiated by serogroup, biotype, and serotype. The serogroups are defined by the surface lipopolysaccharide (LPS) which is composed of three parts: lipid A, core-polysaccharide, and O-polysaccharide (O-PS) (Chatterjee and Chaudhuri, 2003). Among the more than 200 serogroups of *V. cholerae*, which are identified by the O-PS portion of LPS, only two serogroups (O1 and O139) have been shown to be capable of causing cholera epidemics. Within the O1 serogroup, there are two biotypes, classical and El Tor, which differ phenotypically and in disease severity. While the classical biotype was responsible for the first six cholera pandemics, the El Tor biotype is responsible for the ongoing seventh pandemic and has become the predominant circulating biotype worldwide (Bishop and Camilli, 2011; Harris et al., 2012). Among both biotypes, the two most prevalent serotypes of *V. cholerae* are Ogawa and Inaba, which differ by a 2-O-methyl group on the non-reducing terminal sugar of the O-PS that is present in Ogawa and absent in the Inaba serotype (Villeneuve et al., 1999).

There are currently two licensed oral cholera vaccines in use worldwide. Dukoral® is composed of a combination of whole cell killed *V. cholerae* O1 strains, representing both biotypes and

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Ogawa and Inaba serotypes, as well as the recombinant B subunit of cholera toxin (CTB). Shanchol™ contains representative strains of both O1 and O139 serogroups but lacks CTB (Bishop and Camilli, 2011). While the vaccines are safe, they are only moderately effective, in that there is a limited duration of immunity (<3 years), they require multiple doses, and they are not especially effective in young children, a population particularly vulnerable to disease. For these reasons, there are ongoing studies aimed at better understanding the serum and mucosal antibody responses to *V. cholerae* and then applying this information to vaccine development (Pasetti and Levine, 2012).

Serum LPS-specific IgG titers and vibriocidal activity are the two primary measures of immunity to *V. cholerae*. Both factors are important in the assessment of vaccine efficacy, though mucosal (not serum) antibodies are likely the principle mediators of intestinal immunity to *V. cholerae* (Winner et al., 1991; Apter et al., 1993; Harris et al., 2009; Johnson et al., 2012). A particular challenge associated with the analysis of LPS-specific serum antibody titers is the lack of a common IgG standard. Currently, serum antibody levels are compared to pooled human polyclonal antibody preparations from milk or sera (Qadri et al., 1999). Alternatively, baseline titers from healthy human controls are used as a reference, which can be problematic in areas where cholera is endemic and exposure to *V. cholerae* is common (Johnson et al., 2012). While these comparisons allow for relative antibody titer differences to be analyzed within a sample population, it limits comparisons across different clinical studies or vaccine trials. A universal human IgG antibody standard directed against one or more immunodominant epitopes on *V. cholerae* LPS would be of enormous benefit to the cholera research community.

Mouse monoclonal IgA antibodies (mAbs) 2D6 and ZAC-3 bind distinct immunodominant epitopes on *V. cholerae* LPS (Winner et al., 1991; Lullau et al., 1996; Wang et al., 1998). 2D6 IgA recognizes the Ogawa O-polysaccharide antigen defined by 2-O-methyl group on the non-reducing terminal sugar. ZAC-3 IgA recognizes the core/lipid A moiety of Ogawa and Inaba lipopolysaccharides and is thought to be similar to a number of other mAbs like 72.1 that have been shown to be protective in mice against experimental *V. cholerae* infection (Winner et al., 1991; Lullau et al., 1996; Wang et al., 1998; Dharmasena et al., 2009). In this study we produced chimeric mouse-human derivatives of mAbs 2D6 and ZAC-3 in which the V_H and V_L domains of each mAb were grafted onto a human IgG_1 framework. The resulting chimeric antibodies were expressed in *Nicotiana benthamiana*-based rapid antibody-manufacturing platform (RAMP). We demonstrate that the chimeric IgG mAbs retain binding specificities and functional activities associated with the murine mAbs. Because RAMP enables ready, easy to scale antibody production, we propose that chimeric 2D6 and/or ZAC-3 IgG_1 may prove to be valuable standards in cholera vaccine community.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The O1 classical *V. cholerae* O395 strain was a gift from Dr. John Mekalanos (Harvard Medical School) (Mekalanos et al., 1979) and the *V. cholerae* O1 El Tor strain (C6706) was kindly provided by Dr. Fitnat Yildiz (University of California, Santa Cruz). Reference vaccine strain 9459 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Strains were grown in LB medium at 37 °C with aeration (150 rpm) supplemented when necessary with streptomycin (100 μg/ml).

2.2. B cell hybridomas and production of chimeric IgG1 anti-*V. cholerae* mAbs

The 2D6 B cell hybridoma was obtained from Dr. Marian Neutra (Children's Hospital Boston). The ZAC-3 B cell hybridoma was obtained from Dr. Blaise Corthésy (CHUV, Switzerland). The hybridomas were maintained in RPMI medium supplemented with 10% fetal bovine serum (FBS) without antibiotics at 37 °C in a 5% CO_2–95% air atmosphere, as described (Forbes et al., 2008). The murine V_L and V_H domains of 2D6 and ZAC-3 were amplified by PCR from cDNA derived from the respective murine B cell hybridomas (Winner et al., 1991; Lullau et al., 1996). PCR amplicons were sequenced and consensus contigs for each domain were generated based on the Kabat and IMGT databases (Fig. S1–2) (Lefranc, 2009). The codon-optimized V_L and V_H regions of each mAb were then synthesized commercially (GeneArt, LifeTechnologies, Grand Island, NY) and fused to human IgG_1 and κ constant regions (O'Hara et al., 2012; Sully et al., 2014). Chimeric antibodies were expressed using *N. benthamiana*-based rapid antibody-manufacturing platform (RAMP) (Whaley et al., 2011). For this particular study, production was performed with a transgenic *N. benthamiana* line devoid of xylosyl transferase and fucosyl transferase activities, which results in transgenic immunoglobulins with glycans that are generally more homogeneous than those produced in mammalian cells (Schahs et al., 2007). Purity of the chimeric antibodies was assessed by HPLC (data not shown) and SDS-PAGE (Fig. S3). Fab fragments were generated using the IgG Fab preparation kit (ThermoScientific, Rockford, IL) and visualized by SDS-PAGE and GelCode Blue (ThermoScientfic) (Fig. S3). Concentration of Fab fragments was determined by NanoDrop (ThermoScientific, Wilmington, DE).

2.3. ELISAs for determining chimeric mAb specificity

Nunc Maxisorb F96 microtiter plates (ThermoFisher Scientific, Pittsburgh, PA) were coated overnight with poly-L-lysine (10 μg/ml). Overnight cultures of *V. cholerae* O395 (O1 classical Ogawa strain), C6706 (O1 El Tor Inaba strain) or ATCC 9459 (vaccine Inaba strain) were washed twice with PBS, and then applied (50 μl) to poly-L-lysine coated wells. Plates were subjected to centrifugation (1500 rcf for 6 min) and cells were then fixed with 2% paraformaldehyde for 20 min. Residual paraformaldehyde was quenched by the addition of 0.1 M glycine (50 μl) followed by incubation at room temperature for 30 min. Plates were blocked with 2% goat serum overnight at 4 °C before being probed with the chimeric IgG mAbs. Horseradish peroxidase (HRP)-labeled goat anti-mouse IgA-specific polyclonal antibodies and goat anti-human IgG-specific polyclonal antibodies (SouthernBiotech, Birmingham, AL) were used as the secondary reagents. The ELISA plates were developed using the colorimetric detection substrate 3,3′,5,5′-tetramethylbenzidine (TMB; Kirkegaard & Perry Labs, Gaithersburg, MD) and were

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