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Research Paper

Neutralization Takes Precedence Over IgG or IgA Isotype-related Functions in Mucosal HIV-1 Antibody-mediated Protection

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ABSTRACT

HIV-1 infection occurs primarily through mucosal transmission. Application of biologically relevant mucosal models can advance understanding of the functional properties of antibodies that mediate HIV protection, thereby guiding antibody-based vaccine development. Here, we employed a human *ex vivo* vaginal HIV-1 infection model and a rhesus macaque *in vivo* intrarectal SHIV challenge model to probe the protective capacity of monoclonal broadly-neutralizing (bnAb) and non-neutralizing Abs (nnAbs) that were functionally modified by isotype switching. For human vaginal explants, we developed a replication-competent, secreted NanoLuc reporter virus system and showed that CD4 binding site bnAbs b12 IgG1 and CH31 IgG1 and IgA2 isoforms potently blocked HIV-$1_{JR\text{-}CSF}$ and HIV-1_{Bal26} infection. However, IgG1 and IgA nnAbs, either alone or together, did not inhibit infection despite the presence of FcR-expressing effector cells in the tissue. In macaques, the CH31 IgG1 and IgA2 isoforms infused before high-dose SHIV challenge were completely to partially protective, respectively, while nnAbs (CH54 IgG1 and CH38 mIgA2) were non-protective. Importantly, in both mucosal models IgG1 isotype bnAbs were more protective than the IgA2 isotypes, attributable in part to greater neutralization activity of the IgG1 variants. These findings underscore the importance of potent bnAb induction as a primary goal of HIV-1 vaccine development.

Abbreviations: ADCC, Antibody-dependent cell-mediated cytotoxicity; ARV, Anti-retroviral; AZT, Zidovudine; bnAb, Broadly neutralizing antibody; CD4bs, CD4 binding site; dIgA, Dimeric IgA; FcRn, Neonatal Fc receptor; GalCer, Galactosyl ceramide; GI, Gastrointestinal; GU, Genitourinary; IDV, Indinivir; IMC, Infectious molecular clone; LED, Lowest effective dose; mAb, Monoclonal antibody; mIgA, Monomeric IgA; MPER, Membrane-proximal external region; nnAb, Non-neutralizing antibody; pIgR, Polymeric IgA receptor; SC, Secretory component; snLuc, Secreted nanoluciferase; sIgA, Secretory IgA; T/F, Transmitted/founder.

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1. Introduction

HIV-1 transmission occurs primarily through virus infection of genitourinary (GU) or gastrointestinal (GI) mucosae following sexual exposure (UNAIDS, 2014a, UNAIDS, 2014b, UNAIDS, WHO in Partnership with UNICEF and UNAIDS, 2014). Biomedical interventions that can act at portals of HIV-1 entry may be most effective at preventing infection, thwarting the initial seeding of the viral reservoir and limiting systemic spread. Successful immune-based approaches will likely require potent vaccine-induced antibodies (Abs) or passively administered Abs that, in concert with local immune responses, can rapidly defend against mucosal infection. Thus, understanding the properties and functions of Ab-mediated protection against mucosal HIV-1 infection is key in designing effective, multi-layered defense strategies against mucosal transmission of HIV-1.

The potential protective role of broadly reactive, potent anti-Env neutralizing Abs (bnAbs) against HIV-1 infection at mucosal sites has been recognized for decades (Klein et al., 2013a, Moldt et al., 2012, Shingai et al., 2014, Liu et al., 2016, Hessell et al., 2009b, Barouch et al., 2013, Pegu et al., 2014, Stamatatos et al., 2009, Mascola and Haynes, 2013, Hessell et al., 2009a, Mascola et al., 2000, Veazey et al., 2003), but as yet no candidate vaccine tested has elicited these Abs in the circulation of HIV-1-uninfected persons. By contrast, broadly neutralizing Abs (bnAbs) emerge in about 50% of HIV-infected persons within two years of infection (Stamatatos et al., 2009, Hraber et al., 2014), and potent human monoclonal Abs (mAbs) of various epitope specificities that are capable of neutralizing >90% of circulating HIV-1 strains have been generated from memory B cells of HIV-infected persons (Mascola and Haynes, 2013, Burton and Mascola, 2015, Walker et al., 2011, Falkowska et al., 2012, Wu et al., 2010). These bnAbs, delivered as passive immunoprophylaxis, are undergoing clinical evaluation for prevention against HIV-1 acquisition (Caskey et al., 2015, Ledgerwood et al., 2015) after promising results in non-human primate (NHP) studies (Klein et al., 2013a, Pegu et al., 2014, Shingai et al., 2014). Findings in recent years indicate that non-neutralizing Abs (nnAbs) may also exert significant anti-HIV functions (Milligan et al., 2015, Barouch et al., 2015) facilitated through Fc-mediated interactions with FcRs expressed on innate cells in the mucosa (Alter and Moody, 2010, Li et al., 2014, Chung et al., 2015). For example, anti-V1V2 IgG Abs capable of mediating Ab-dependent cellular cytotoxicity (ADCC) were induced by the candidate HIV-1 vaccine regimen in the RV144 trial (Pollara et al., 2014), which at relatively high levels correlated with reduced infection risk (Haynes et al., 2012, Yates et al., 2014). These observations provided optimism that vaccine-induced non-neutralizing, type-specific Abs may indeed contribute substantially to vaccine-induced protection and could be a more achievable goal for vaccine designs (Haynes and McElrath, 2013, Barouch et al., 2015). Thus, defining the key roles and comparing the efficacy of bnAb *vs.* nnAb functions in relevant models has been a major focus of recent investigations.

A surprising finding in the RV144 immune correlates analysis was that monomeric anti-Env IgA in plasma may mitigate otherwise protective IgG responses (Haynes et al., 2012, Tomaras et al., 2013). Both IgA and IgG antibody isotypes are key mediators of defense against pathogen invasion. Although IgG is more abundant in the lower female genital tract and male foreskin epidermis, IgA is the main Ab isotype in most mucosal compartments (Lemos et al., 2016, Mestecky et al., 2009). The two human subclasses of IgA, IgA1 and IgA2, can each exist as dimeric (dIgA) and multimeric forms comprising two or more monomeric IgAs covalently linked by a J-chain; multimerization enhances IgA avidity and its aggregation potential (Stieh et al., 2014, Woof and Russell, 2011). Compared to IgA1, IgA2 is the more prevalent subclass in the female genital tract and colon (Woof and Russell, 2011), and its shorter hinge region and additional disulfide bonds create a more compact, rigid structure with enhanced resistance to degradation by proteases present in the mucosa. The polymeric IgA receptor (pIgR) provides unidirectional transport of dIgA to the apical epithelial surface, where it complexes with secretory component (SC), resulting in secretory IgA (sIgA). Moreover, the glycosylation of IgA SC facilitates the interaction with mucus (Woof and Russell, 2011). Transport and maintenance of IgG in the GI and GU tracts are regulated by the neonatal Fc receptor (FcRn) on epithelial cells, which binds IgG at low pH and releases it at neutral pH (Pyzik et al., 2015, Tzaban et al., 2009). In the lower female genital tract, the acidic pH of the lumen favors retention of IgG at this site. In the gut, FcRn enables transport of IgG to the lumen where it can bind to its cognate antigen and then return as immune complexes for presentation to dendritic cells (Pyzik et al., 2015, Tzaban et al., 2009). Thus, FcRn may paradoxically also enable transcytosis of infectious HIV-1 virions carried in tow by IgG (Gupta et al., 2013). In summary, differences in the structure, transport and Fc-mediated effector functions can influence the role of IgG and IgA Abs in mucosal HIV-1 infection.

Despite its importance in mucosal host defense, the role of IgA in mucosal protection against HIV-1 transmission is less defined than that of IgG. To address this, we generated a mAb panel of IgG1 and various IgA isoforms, with particular emphasis on the more stable IgA2 subclass; these include a bnAb directed against the HIV-1 gp120 CD4 binding site (CD4bs) neutralizing epitopes, and nnAbs directed at gp120 C1 and V2 epitopes and the gp41 immunodominant domain (Bonsignori et al., 2011, Wu et al., 2011, Liu et al., 2013, Liao et al., 2013, Bonsignori et al., 2012). Moreover, we have expressed the CD4bs bnAb, CH31, as IgG1, and as monomeric, dimeric and secretory IgA2 (Zhang et al., 2016). We similarly expressed the IgG1, monomeric and dimeric IgA2 isoforms of the non-neutralizing gp41-specific mAb, 7b2 (Zhang et al., 2016, Santra et al., 2015). Using these mAbs, the impact of various IgA forms on the functional capacity of neutralizing and non-neutralizing mAbs was investigated in various assays and models of HIV-1 transmission. This panel has been extensively characterized in a variety of *in vitro* assays supporting their potential to exert various antiviral functions *in vivo*, such as phagocytosis (Tay et al., 2016), virus capture (Liu et al., 2013, Liu et al., 2014), virus aggregation (Stieh et al., 2014, R. Shattock, personal communication), blocking of virus binding to galactosyl ceramide (GalCer) (Dennison et al., 2014), ADCC (Tomaras et al., 2013, Pollara et al., 2014, Bonsignori et al., 2012) and neutralization (Santra et al., 2015). Here, we have evaluated the protective capacity of these mAbs *ex vivo* in a human vaginal tissue explant model and *in vivo* in an NHP intrarectal model of HIV-1 infection to identify key Ab properties associated with early mucosal protection that may guide the development of effective prevention strategies.

2. Materials and Methods

2.1. Ethics

Indian-origin rhesus monkeys used in the immunization studies were housed and maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited institution in accordance with the principles of the National Institute of Health. All studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in BIOQUAL (Rockville, MD). BIOQUAL is fully accredited by AAALAC and through OLAW, Assurance Number A-3086. The animal protocol used in this study was approved by the BIOQUAL IACUC (#14-B080). All physical procedures associated with this work were done under anesthesia to minimize pain and distress in accordance with the recommendations of the Weatherall report, "The use of non-human primates in research". Teklad 5038 Primate Diet was provided once daily by animal size and weight. The diet was supplemented with fresh fruit and vegetables. Fresh water was given *ad libitum*.

Human vaginal tissue, that would otherwise have been discarded, was obtained from healthy women (free of known malignancy) undergoing vaginal repair surgeries in the Department of Obstetrics and Gynecology at the University of Washington. These surgical remnants