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Research paper

Vaccine-induced plasmablast responses in rhesus macaques: Phenotypic characterization and a source for generating antigen-specific monoclonal antibodies

Eduardo L. V. Silveira [a,b,c], Sudhir P. Kasturi [b], Yevgeniy Kovalenkov [a], Ata Ur Rasheed [b], Patryce Yeiser [a], Zarpheen S. Jinnah [b,c], Traci H. Legere [b,c], Bali Pulendran [b,d], Francois Villinger [b,c,d], Jens Wrammert [a,b,*]

[a] Department of Pediatrics and Department of Hematology and Oncology, Emory University School of Medicine, Atlanta, GA 30322, USA
[b] Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA
[c] Division of Microbiology and Immunology, Yerkes National Primate Research Center, Emory University, Atlanta, GA, 30329, USA
[d] Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, 30322, USA

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ABSTRACT

Over 100 broadly neutralizing antibodies have been isolated from a minority of HIV infected patients, but the steps leading to the selection of plasma cells producing such antibodies remain incompletely understood, hampering the development of vaccines able to elicit them. Rhesus macaques have become a preferred animal model system used to study SIV/HIV, for the characterization and development of novel therapeutics and vaccines as well as to understand pathogenesis. However, most of our knowledge about the dynamics of antibody responses is limited to the analysis of serum antibodies or monoclonal antibodies generated from memory B cells. In a vaccine setting, relatively little is known about the early cellular responses that elicit long-lived plasma cells and memory B cells and the tools to dissect plasmablast responses are not available in macaques. In the current study, we show that the majority (>80%) of the vaccine-induced plasmablast response are antigen-specific by functional ELISPOT assays. While plasmablasts are easily defined and isolated in humans, those same phenotypic markers have not been useful for identifying macaque plasmablasts. Here we describe an approach that allows for the isolation and single cell sorting of vaccine-induced plasmablasts. Finally, we show that isolated plasmablasts can be used to efficiently recover antigen-specific monoclonal antibodies through single cell expression cloning. This will allow detailed studies of the early plasmablast responses in rhesus macaques, enabling the characterization of both their repertoire breadth as well as the epitope specificity and functional qualities of the antibodies they produce, not only in the context of SIV/HIV vaccines but for many other pathogens/vaccines as well.

1. Introduction

While more than 30 years has passed since the discovery of HIV as the etiology of AIDS, there is no efficient vaccine available yet. Initial efforts to develop a vaccine against HIV were directed towards generating antibody-mediated responses, but as the virus could readily escape from them, the HIV vaccine field turned largely in the direction of T cell-mediated vaccine development (reviewed by Koup and Douek, 2011). However, recent progress dissecting B cell responses in chronically HIV infected patients has led to the identification and analysis of several broadly neutralizing antibodies (bnAbs) that eventually develop in a small fraction of patients

* Corresponding author at: Department of Pediatrics and Department of Hematology and Oncology, Emory University School of Medicine, HSRB building Rm E480, 1760 Haygood Dr. Atlanta, GA 30322, USA. Tel.: +1 404 778 3265.
E-mail address: jwramme@emory.edu (J. Wrammert).

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(reviewed by West et al., 2014). These antibodies display a remarkable breadth of neutralization, appear late in infection (reviewed by Haynes et al., 2012) and are specific for several different epitopes of Env gp120 or gp41 (Walker et al., 2009). As a group, these bnAbs often share certain unusual attributes such as long CDR3 regions, extremely high levels of somatic hypermutation and polyreactivity against self and non-self antigens (Liao et al., 2011; West et al., 2014). These broadly neutralizing antibodies can prevent simian/human immunodeficiency (SHIV) virus infection in a macaque model after passive immunization (Hessell et al., 2009), and their therapeutic administration has been shown to reduce viral titers to undetectable levels, comparable to highly active antiretroviral therapy (HAART) (Barouch et al., 2013; Shingai et al., 2013). Even though recent papers (Liao et al., 2013; Doria-Rose et al., 2014; Fera et al., 2014) have elegantly described the evolution of these broadly neutralizing antibodies in concert with the evolution of the virus, from the early to a late chronic stage of infection, it still remains an open question if and indeed how a vaccine can be designed that can induce similar responses.

In order to design novel vaccines that are able to induce B cell responses focused on the epitopes targeted by these broadly neutralizing antibodies, both new and improved immunogens are needed, as well as a better understanding of the early B cell responses induced by these novel vaccine candidates (Burton et al., 2012). One way to study these early B cell responses is through the use of antigen-probes designed to stain antigen-specific memory B cells (Scheid et al., 2009b; Franz et al., 2011; Kardava et al., 2014). This approach has proven to be very powerful in order to identify the bnAbs described above (Scheid et al., 2009a; Walker et al., 2011; Sundling et al., 2012a). Another attractive route to characterize the early B cell responses is through the analysis of plasmablasts appearing in the peripheral blood as a consequence of vaccination (Wrammert et al., 2008; Lee et al., 2011; Liao et al., 2011; Li et al., 2012) or infection, such as HIV (Doria-Rose et al., 2009; Liao et al., 2011), influenza (Wrammert et al., 2011), dengue (Wrammert et al., 2012), cholera (Rahman et al., 2013), respiratory syncytial virus (RSV) (Lee et al., 2010) and nosocomial bacteria (Band et al., 2014). During a recall response, human plasmablasts numbers peak around 7 days post-vaccination (Wrammert et al., 2008; Mei et al., 2009; Li et al., 2012) with a preference for IgG- or IgA-secreting cells, suggesting a memory B cell-derived origin. This notion is also supported by a very high level of somatic hypermutation in these cells. Furthermore, the magnitude of the plasmablast response has been shown to correlate directly with the induction of neutralizing antibody titers and thus the potential to clear infection (Balakrishnan et al., 2011; Nakaya et al., 2011; Li et al., 2012). Finally, since the majority of plasmablasts are antigen-specific at the peak of their response, they represent an excellent source of material to produce antigen-specific monoclonal antibodies (mAbs). The characterization of those mAbs in terms of repertoire breadth, binding affinity, epitope specificity and neutralizing activity provides a snapshot of the early ongoing antibody response at a single cell level (Wrammert et al., 2008, 2011; Li et al., 2012; Xu et al., 2012; Nakamura et al., 2013).

Rhesus macaques have emerged as a major model for studies of HIV (or SIV and SHIV) pathogenesis, evaluation of novel therapeutics or antivirals, as well as for vaccine development (Hansen et al., 2013; Ling et al., 2013; Zhou et al., 2013; Roederer et al., 2014). In addition, this model is widely used to investigate many other human pathogens as well as the efficacy of new vaccine candidates (reviewed by Gujer et al., 2011). Although several studies have evaluated B cells in animal models for HIV infection or vaccination, plasmablast responses in macaques remain poorly characterized, both in terms of the kinetics of their appearance after vaccination and suitable phenotypic markers to identify them. In one study, plasmablast responses could be detected by ELISPOT in macaque peripheral blood 7 days after vaccination, similar to what is observed in humans, however a detailed kinetic analysis was not performed (Sundling et al., 2010). Another publication showed that plasmablasts could be detected 2 weeks after booster immunization during ART treatment in macaques chronically infected with SIV (Demberg et al., 2012), however it is unclear how these results would translate into that of a vaccinated, non-infected host. In addition, a lack of suitable surface markers to identify and isolate functional, non-permeabilized plasmablasts in macaques, have hampered their further characterization. Finally, the relatively poor annotation of the macaque Ig locus, as compared to the human counterpart, has also been a major obstacle. However, recent progress has been made in annotating the macaque Ig locus and designing primers for amplification of macaque single cell Ig rearrangements (Sundling et al., 2012b) leading to an efficient isolation of CD4 binding site specific antibodies from macaques vaccinated with an HIV antigen (Sundling et al., 2014).

In this study, we characterize antigen-specific plasmablast responses in macaque peripheral blood after booster immunizations with SIV gp140 and gag proteins combined with a potent adjuvant that consists of poly(lactic-co-glycolic) acid (PLGA) based nanoparticles (NP adjuvant) containing TLR4 and TLR7/8 agonists (MPL and R848), respectively (Kasturi et al., 2011). This vaccination induces a plasmablast response that appears rapidly, even earlier than in humans, and almost all these cells are antigen-specific. Phenotypic characterization of these vaccine-induced plasmablasts, in combination with cell sorting and functional readouts, allowed for their isolation as either bulk or single cells. The isolated bulk cells were used to confirm their phenotype by functional ELISPOT assays, while the single cells were used both for repertoire analysis and for generation of monoclonal antibodies. About half of the mAbs produced in this fashion were SIV gp140-specific, clearly illustrating the efficacy of this approach. The establishment of this technology for use in macaques will provide a novel tool for analysis of vaccine- or infection-induced plasmablast responses and a better understanding of the antibodies that these cells produce.

2. Material and methods

2.1. Animals and Immunizations

Rhesus macaques (*Macaca mulatta*) of Indian origin were housed at Yerkes National Primate Research Center. The animals were cared for in conformance to the guidelines of the Committee on the Care and Use of Laboratory Animals ((U.S.), 1996). All experimental protocols and procedures were reviewed and approved by the Emory Institutional Animal Care and Use Committee.