EBioMedicine 18 (2017) 216–224

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Comprehensive Characterization of Humoral Correlates of Human Immunodeficiency Virus 1 Superinfection Acquisition in High-risk Kenyan Women

Keshet Ronen [a,b,c,1], Adam S. Dingens [a,c,d,1], Susan M. Graham [b,c,e], Walter Jaoko [f], Kishor Mandaliya [b], R. Scott McClelland [b,c,e,g], Julie Overbaugh [a,h,*]

[a] Human Biology Division, Fred Hutch Cancer Research Center, 1100 Fairview Ave N., Seattle, WA 98109, USA
[b] Department of Global Health, University of Washington, 325 9th Avenue, Seattle, WA 98104, USA
[c] Department of Epidemiology, University of Washington, 1959 NE Pacific Street, Seattle, WA 98195, USA
[d] Molecular and Cellular Biology Program, University of Washington, 1959 NE Pacific Street, Seattle, WA 98195, USA
[e] Department of Medicine, University of Washington, 1959 NE Pacific Street, Seattle, WA 98195, USA
[f] Department of Medical Microbiology, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya
[g] Institute of Tropical and Infectious Disease, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya
[h] Public Health Sciences Division, Fred Hutch Cancer Research Center, 1100 Fairview Ave N., Seattle, WA 98109, USA

ARTICLE INFO

Article history:
Received 30 January 2017
Received in revised form 27 March 2017
Accepted 3 April 2017
Available online 7 April 2017

Keywords:
HIV
Superinfection
Antibody
Neutralization
Binding
Immune correlate

ABSTRACT

HIV-1 superinfection, in which an infected individual acquires a second HIV-1 infection from a different partner, is one of the only settings in which HIV acquisition occurs in the context of a pre-existing immune response to natural HIV infection. There is evidence that initial infection provides some protection from superinfection, particularly after 6 months of initial infection, when development of broad immunity occurs. Comparison of the immune response of superinfected individuals at the time of superinfection acquisition to that of individuals who remain singly infected despite continued exposure can shed light on immune correlates of HIV acquisition to inform prophylactic vaccine design. We evaluated a panel of humoral immune responses in the largest published group of superinfected individuals (n = 21), compared to a set of 3:1 matched singly infected controls from the same cohort. The immune functions studied included plasma neutralization, plasma and cervical antibody-dependent cellular cytotoxicity, and plasma IgG and IgA binding to a panel of 18 envelope antigens, including correlates of HIV acquisition in the RV144 vaccine trial, IgG binding to V1V2 and IgA binding to gp140. Association between each immune function and HIV superinfection was evaluated using conditional logistic regression. No significant associations were detected between any of the immune functions and superinfection acquisition. This study constitutes the most comprehensive and detailed characterization of multiple immune correlates of superinfection to date. The results suggest that immune responses not commonly measured in current HIV studies may be important in protection from HIV infection, and these or a more robust humoral response than that seen in naturally infected women may be needed for a protective vaccine.

1. Introduction

In 2015, 2.1 million people were newly infected with HIV-1 globally (UNAIDS, 2016). While growing numbers have access to treatment and significant advances have been made in prevention (UNAIDS, 2016), a protective vaccine is essential to ending the HIV pandemic. However, development of an efficacious prophylactic HIV vaccine has been enormously challenging. An important barrier to HIV vaccine development has been the field's limited understanding of the nature of protective HIV-specific immunity. Recent analysis of participants in the RV144 HIV vaccine trial, which showed modest (~31%) efficacy, identified several humoral correlates of acquisition among vaccine recipients (Haynes et al., 2012). HIV acquisition was inversely correlated with plasma IgG binding to the V1V2 portion of the HIV envelope protein (Env), and directly correlated with IgA binding to Env. No significant effects of neutralizing antibody responses were detected. The results of this hypothesis-generating study have prompted validation of these correlates of acquisition risk in other settings.

Apart from vaccine studies, superinfection (SI), in which an HIV-infected individual acquires a second HIV infection, is one of the only settings in which HIV exposure occurs in the presence of a pre-existing

* Corresponding author at: Human Biology Division, Fred Hutch Cancer Research Center, 1100 Fairview Ave N., Seattle, WA 98109, USA.
E-mail address: joverbau@fredhutch.org (J. Overbaugh).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.ebiom.2017.04.005

HIV-specific immune response. SI has been documented in a number of settings (Altfeld et al., 2002; Smith et al., 2004; Chohan et al., 2005; Piantadosi et al., 2007; Redd et al., 2012; Ronen et al., 2013). Early epidemiologic studies in several high-risk cohorts suggested the incidence of SI was similar to that of initial infection (Smith et al., 2004; Kraft et al., 2010; Redd et al., 2013). However, analysis of a larger cohort of SI cases ($n = 21$) from the Mombasa Cohort, a cohort of high-risk women in Mombasa, Kenya, suggests that HIV-infected individuals are partially protected from SI: the incidence of SI was roughly half that of initial infection in the cohort after adjustment for differences in risk behavior, with evidence of greater protection after 6 months of infection, suggesting a potential immune mechanism (Ronen et al., 2013). Comparison of immune responses immediately before SI acquisition with responses among individuals exposed to HIV who do not acquire SI is therefore a promising strategy to identify immune correlates of HIV acquisition. Importantly, these correlates may differ from correlates of virologic control after establishment of chronic infection.

To date, no studies have examined the immune correlates identified in the RV144 trial for their possible role in protection against SI. Moreover, studies that have investigated other humoral correlates of SI, which have had conflicting findings, have generally been limited to examining one response at a time and small numbers of cases. Two case-controls studies in the Mombasa Cohort found no difference between cases and singly-infected controls: one included 6 SI cases and investigated neutralizing antibody breadth and potency (Blish et al., 2008; UNAIDS, 2016) and the other included 12 SI cases and investigated antibody-dependent cellular viral inhibition (ADCVI) (Haynes et al., 2012; Forthal et al., 2013). However, studies in other cohorts, each comparing 3 SI cases to singly-infected controls, have suggested lower autologous neutralizing antibody (Altfeld et al., 2002; Smith et al., 2004; Chohan et al., 2005; Smith et al., 2006; Piantadosi et al., 2007; Basu et al., 2012; Redd et al., 2012; Ronen et al., 2013) and antibody-dependent cellular cytotoxicity (ADCC) (Basu et al., 2014) in SI cases than controls. Given these conflicting findings, larger studies are needed. Additionally, because synergistic or antagonistic relationships likely exist between different immune functions, any correlate of protection may in fact consist of a combination of responses, which would be missed in studies of a single immune measure. Indeed, interaction analysis in the RV144 study showed that in the presence of low levels of Env-specific IgA, ADCC activity and infection risk were inversely associated, while in the presence of high Env-specific IgA, no such association was present (Haynes et al., 2012). This finding has been hypothesized to be due to competition between binding antibodies (Tomaras et al., 2013). Evaluating multiple immune correlates in aggregate may provide a more nuanced understanding of HIV acquisition risk.

Here, we present a comprehensive analysis of an array of humoral immune responses in 21 SI cases from the Mombasa Cohort compared to individually-matched singly infected controls from the same cohort. This study constitutes the most detailed characterization of multiple immune correlates of SI in the largest group of SI cases analyzed to date.

2. Materials and Methods

2.1. Study Population and Sample Selection

Subjects were drawn from the Mombasa Cohort, an ongoing prospective cohort of initially HIV-negative high-risk women in Mombasa, Kenya, as previously described (Martin et al., 1998). All participants provided written informed consent. Ethical approval was provided by the ethical review boards of the University of Nairobi, University of Washington and Fred Hutchinson Cancer Research Center. Twenty-one SI cases were identified by subjecting samples collected from 146 HIV-1 infected women with well-defined initial infection dates to screening by 454 pyrosequencing or Sanger sequencing, as previously described (Chohan et al., 2005; Piantadosi et al., 2007, 2008; Ronen et al., 2013). SI was detected based on evidence of a phylogenetically distinct viral variant at a timepoint after initial infection. Timing of SI was estimated as the midpoint between the first timepoint in which the SI variant was detected and the last singly infected timepoint. All participants were antiretroviral naïve at the time of sampling. Antiretroviral therapy became available in 2004, after which it was offered to eligible patients in accordance with World Health Organization and Kenyan Ministry of Health guidelines. Participant followup included quarterly sampling of plasma and cervical swabs. Cervical swabs were stored in Fetal Bovine Serum with 10% DMSO. All samples were frozen at −80 °C within 24 h of collection.

The present study employed a case-control design, and examined immune functions in both plasma and cervical swab supernatants to assess systemic responses and responses at the likely mucosal site of HIV exposure in this sex worker cohort. From the 21 identified SI cases, 20 plasma specimens and 17 cervical swab supernatants were available at the last available singly infected timepoint. For each case sample, 3 singly-infected control samples of the same specimen type were selected, matched to each SI case based on initial infection viral subtype in the *env* gene and on time since initial infection (±30 days). All controls were HIV-infected women who seroconverted after enrollment in the Mombasa Cohort and were screened for and had no evidence of SI.

2.2. Plasma and Cervical IgG Titer

IgG ELISA was performed as described in (Williams et al., 2015). In brief, Immunolon 2-HB plates were coated with 2500 ng of goat anti-human antibody (Sigma) diluted in 0.1 M sodium bicarbonate coating buffer (pH 7.4) overnight at 4 °C. Plates were washed with PBS-0.05% Tween and blocked with 10% non-fat dry milk (NFDM) diluted in PBS-0.05% Tween. After at least 1 h, the milk block was removed, and 100 μL of plasma or cervical samples diluted in NFDM added for 1 h at 37 °C. Samples were serially 10-fold diluted to identify the end-point titer: plasma dilutions spanned 1 in 10^4 to 1 in 10^6; cervical sample dilutions spanned 1 in 10^2 to 1 in 10^5. Plates were washed after sample incubation, and 100 μL anti-human-IgG-HRP (Sigma), diluted in 1:2500 in NFDM added and incubated for 1 h at room temperature. Plates were and 50 μL Ultra-TMB (Thermo Scientific) substrate was added for 10 min. The reaction was stopped by adding an equal volume of 0.1 M H_2SO_4 and the absorbance read within 30 min using 450 nM optical density. The endpoint titer was defined as the average Ab concentration with binding greater than double the binding by NFDM only.

2.3. Neutralization Assays

To score neutralizing antibody activity, we used a previously published 4-pseudovirus panel (Cortez et al., 2015). This panel was constructed to include diverse envelope variants isolated early in infection whose neutralization profiles could provide a range of breadth and potency scores (scoring described below). We first identified variants whose IC50 values varied across individuals and were predictive of NAb breadth scores generated using larger pseudovirus panels in previous studies in the Mombasa Cohort (Blish et al., 2008; Piantadosi et al., 2009; Cortez et al., 2012). A subset of 4 viruses from these panels was found to be predictive of findings based on the larger panels: Q461.d1 (Tier 1b, subtype A) (Long et al., 2002), QD435.100M.a4 (Tier 2, subtype D) (Blish et al., 2009), Q842.d16 (Tier 2, subtype A) (Long et al., 2002), Du156.12 (Tier 2, subtype C) (Li et al., 2006). For example, in a prior study of breadth at 5 years post initial infection among singly and superinfected individuals (Cortez et al., 2012), superinfected individuals had on average 1.68 (95% CI 1.24–2.26) times greater breadth scores than singly infected controls when using an 8-virus panel. The smaller 4-virus panel estimated 1.65 (95% CI 1.08–2.50) times greater breadth scores in superinfected than singly infected controls.

Pseudoviruses were produced in HEK 293T cells by cotransfecting equimolar concentrations of the cloned *env* gene and Q23Δ*env* (Long et al., 2002), a subtype A full-length proviral clone with a partial deletion in *env*, using Fugene-6. Forty-eight hours post-transfection,