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Research Paper

Evaluation of Different Parameters of Humoral and Cellular Immune Responses in HIV Serodiscordant Heterosexual Couples: Humoral Response Potentially Implicated in Modulating Transmission Rates

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ABSTRACT

As the HIV/AIDS pandemic still progresses, understanding the mechanisms governing viral transmission as well as protection from HIV acquisition is fundamental. In this context, cohorts of HIV serodiscordant heterosexual couples (SDC) represent a unique tool. The present study was aimed to evaluate specific parameters of innate, cellular and humoral immune responses in SDC. Specifically, plasma levels of cytokines and chemokines, HIV-specific T-cell responses, gp120-specific IgG and IgA antibodies, and HIV-specific antibody-dependent cellular cytotoxicity (ADCC) activity were assessed in nine HIV-exposed seronegative individuals (ESN) and their corresponding HIV seropositive partners (HIV^+-P), in eighteen chronically infected HIV subjects (C), nine chronically infected subjects known to be HIV transmitters (CT) and ten healthy HIV^- donors (HD). Very low magnitude HIV-specific cellular responses were found in two out of six ESN. Interestingly, HIV^+-P had the highest ADCC magnitude, the lowest IgA levels and the highest IgG/IgA ratio, all compared to CT. Positive correlations between $CD4^+$ T-cell counts and both IgG/IgA ratios and %ADCC killing uniquely distinguished HIV^+-P. Additionally, evidence of IgA interference with ADCC responses from HIV^+-P and CT is provided. These data suggest for the first time a potential role of ADCC and/or gp120-specific IgG/IgA balance in modulating heterosexual transmission. In sum, this study provides key information to understand the host factors that influence viral transmission, which should be considered in both the development of prophylactic vaccines and novel immunotherapies for HIV-1 infection.

1. Introduction

The pandemic of HIV/AIDS is still a major public health concern worldwide (Frank and Witten, 2016). Highly active antiretroviral therapy (HAART) can diminish viral loads (VL) to undetectable levels, reducing not only mortality but also transmission rates, with the subsequent impact on a population level (Hull et al., 2014). However, it does not cure the infection. Moreover, an effective vaccine has not been developed yet (Deeks et al., 2016).

It is well known the fact that not all HIV-infected individuals progress to AIDS at the same rate. For instance, there is a peculiar population of HIV^+ subjects able to maintain undetectable VL for >10 years in the absence of HAART and AIDS-related diseases. These subjects are known as *Elite Controllers* (ECs). Even though the correlates of protection in these individuals have not been fully identified, it has been suggested that genetic (presence of HLA-B*57, HLA-B*27, HLA-B*13 and HLA-B*58.01) and immune ($CD8^+$ T-cell response) host factors could be involved (McDermott and Koup, 2012; Gonzalo-Gil et al., 2017). Similarly, recent evidence indicated that humoral immunity could mediate protection in this group (Lambotte et al., 2009; Ackerman et al., 2016).

In the same line, there are other individuals that, despite having being exposed to the virus for long periods of time, are resistant to HIV infection (Rowland-Jones and McMichael, 1995). This phenomenon

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was observed among (i) heterosexual partners of HIV infected people (Ranki et al., 1989; Langlade-Demoyen et al., 1994), (ii) sex workers (Rowland-Jones et al., 1995; Fowke et al., 1996), (iii) men who have sex with men, (iv) intravenous drug users, (v) exposed uninfected infants (Rowland-Jones et al., 1993; Cheynier et al., 1992) and, (vi) health-care workers (Clerici et al., 1994; Pinto et al., 1995). The mechanisms that allow these individuals to be "protected" from the virus are still unknown, although several hypotheses have been proposed. To date, only homozygosis for a 32-base pair deletion in the gene encoding the CCR5 protein (CCR5Δ32), the major coreceptor used by viral isolates most frequently associated with transmission events (R5-tropic HIV-1 variants) has been consistently shown to be a determinant of HIV resistance (Liu et al., 1996). Still, subjects bearing the WT *ccr5* gene have been described as resistant or less susceptible to HIV infection. Thus, unraveling this mystery and understanding the underlying mechanism could help in the development of novel therapies and even a vaccine.

Sexual transmission is currently the major route of HIV infection worldwide accounting for >80% of new infections (Hladik and McElrath, 2008). By definition, a HIV serodiscordant couple (SDC) is a couple in which one partner is HIV-positive and the other is HIV-negative. SDC cohorts may be the most relevant groups for identifying correlates of protection affecting sexual transmission. The first evidence of resistance to infection in spite of exposure in this kind of cohorts appeared in 1989, when T-cell responses to HIV proteins were observed in the seronegative partners, later defined as exposed seronegative (ESN) individuals (Ranki et al., 1989). Since then, cellular, humoral and innate immune responses in ESN subjects have been studied (review in (Piacentini et al., 2008)). Remarkably, there are certain aspects of the immune response that have been recently associated with protection from disease progression but have not been investigated in the scenario imposed by SDC yet. This is the case of antibody-dependent cellular cytotoxicity (ADCC). HIV-specific ADCC-mediating antibodies have been found in plasma of HIV-infected individuals (Forthal et al., 2001; Dugast et al., 2014; Cereb et al., 1995), in cervicovaginal fluids (Battle-Miller et al., 2002), breast milk (Mabuka et al., 2012) and semen (Parsons et al., 2016) of infected subjects. Several reports suggest that ADCC-mediating antibodies might protect infected individuals from disease progression (Lambotte et al., 2009; Thobakgale et al., 2012; Baum et al., 1996; Chung et al., 2011). Recently, our group demonstrated that gp120-specific IgA is a plasma factor capable of modifying the magnitude of IgG-mediated ADCC in HIV infection, possibly abrogating its protective role (Ruiz et al., 2016). The presence of antibodies capable of mediating ADCC at sites of viral entry raises the possibility that these antibodies could modulate viral transmission, probably by inhibiting or decreasing transmission rates. In this line, it was reported that passively acquired ADCC activity in infants born to HIV-infected mothers was not associated with protection but with reduced mortality (Milligan et al., 2015).

In sum, HIV transmission is a complex phenomenon (Dale et al., 2013). The group of SDC is a valuable set of subjects that may help us to study not only correlates of protection in ESN, but also the existence of possible factors involved in viral transmission. Here, we aimed to evaluate different aspects of innate, cellular and humoral immune responses in a cohort of SDC. For this, plasma levels of 39 cytokines and chemokines, HIV-specific T-cell responses, gp120-specific IgG and IgA antibodies, and HIV-specific ADCC activity were evaluated in ESN from SDC and their respective HIV^+ partners (HIV^+-P). Also, the same parameters were evaluated in chronically infected HIV^+ subjects (C), chronically infected subjects known to be HIV transmitters (CT) and healthy donors (HD), for comparison purposes. We hypothesized that the joint evaluation of the aforementioned parameters will shed light on possible immune correlates of protection in ESN as well as possible factors affecting viral transmission. Interestingly, ADCC magnitude, gp120-specific IgA titers and gp120-specific IgG/IgA ratio appeared as factors able to discriminate HIV^+-P, CT and C subjects. This warrants further studies on the role of gp120-spcific IgA, ADCC-mediating antibodies and the relation of these two factors in modulating viral transmission.

2. Materials and Methods

2.1. Study Groups

The following study groups were enrolled (Table 1): Nine serodiscordant heterosexual couples (SDC), eighteen chronically infected subjects (C) and nine chronically infected subjects known to be HIV transmitters (CT). Also, ten healthy HIV-seronegative donors (HD) were enrolled as a control group. SDC were enrolled based on self-reporting being a couple for a period longer than three years and having unprotected sex during that period. The HIV^+ individual of the couple (hereinafter HIV^+ positive partner (HIV^+-P)) self-reported being off-HAART throughout that period and had detectable (>50 HIV RNA copies/ml plasma) viral load (VL) at the moment of enrollment. It is important to highlight that these subjects were not followed up by our team, and SDC were enrolled prior to the development of the HIV treatment guidelines which indicate that all individuals with a negative partner should receive treatment as prevention. Seronegative status of the HIV negative subject (from now on exposed seronegative partner, ESN) was corroborated at the moment of enrollment.

C and CT were defined as subjects infected for >3 years, with detectable VL and HAART naïve. In addition, CT had at least one transmission event documented by self-reported, clinical and phylogenetic data (Damilano G, unpublished). Samples from HD were obtained from >18 year-old voluntary blood donors who completed and passed a survey on blood donation which specifically excludes persons who had been exposed to HIV; and were screened for serological markers before being accepted as donors.

Blood samples from HIV-infected individuals, uninfected sexual partners from HIV-infected individuals, and healthy donors were obtained for this study. Prior to enrollment, the study was reviewed and approved by the *Comité de Ética Humana, Facultad de Medicina, Universidad de Buenos Aires.* All participants provided written informed consent accepting to participate in this study.

2.2. Samples

Plasma and peripheral blood mononuclear cells (PBMCs) were obtained from ESN and HIV^+-P (both individuals from the SDC), HD and C. For CT only plasma samples were obtained with the purpose of being used as control group for ADCC assays. In all cases, blood samples were collected and centrifuged to separate plasma, which was stored at −80 °C until use. For ADCC assays, plasma samples were first diluted (10-fold in RPMI medium) passed through a 0.2 μm-pore filter and heat-inactivated (1 h, 56 °C). PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation (GE Healthcare, UK) and cryopreserved for subsequent assays. PBMCs from one HIV negative donor were isolated, cryopreserved and used as effector cells in ADCC assay. Cells from the same donor were used in all assays to avoid bias from donor to donor. Plasma VL (branched-DNA, Versant HIV-1 RNA 3.0 assay; Siemens Healthcare, UK) and $CD4^+$ T-cell count (flow cytometry double platform, BD FACSCanto; BD Biosciences, USA) were assessed in all subjects.

2.3. HIV Subtype Determination

HIV-1 RNA was extracted from 200 μl of stored plasma using the viral RNA extraction Kit (Purelink viral RNA/DNA Mini kit, Invitrogen-Life Technologies). Afterwards, *pol* gene was amplified by RT-PCR as described before (Dilernia et al., 2013). Amplicons were purified and sequenced using the Big Dye Terminator sequencing kit v3.1 (Applied Biosystems, USA) on an automated sequencer (Applied Biosystems DNA sequencer 3500). Nucleotide sequences were analyzed and manually adjusted using Sequencher v5.1 software (Gene Codes Co.). The partial *pol* sequences were aligned independently of each other with HIV-1

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